AD

IRI Report No. 2588

HMX: TOXICOKINETICS OF ^{14}C-HMX FOLLOWING ORAL ADMINISTRATION TO THE RAT AND MOUSE AND INTRAVENOUS ADMINISTRATION TO THE RAT

Final Report by:

B.D. Cameron

March 1986

Supported by:

U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, Maryland, 21701

Contract No. DAMD-17-80-C-0053
IRI Project 415669PK

Inveresk Research International Limited
Musselburgh, EH21 7UB, Scotland

Contracting Officer's Technical Representative:

Jesse J. Barkley, Jr.
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

Approved for public release; distribution unlimited

Non-classified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A171 600	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) HMX: Toxicokinetics of ^{14}C-HMX following oral administration to the rat and mouse and intravenous administration to the rat		5. TYPE OF REPORT & PERIOD COVERED Final
		6. PERFORMING ORG. REPORT NUMBER 415669 PK/2588
7. AUTHOR(s) B.D. Cameron		8. CONTRACT OR GRANT NUMBER(s) DAMD-17-80-C-0053
9. PERFORMING ORGANIZATION NAME AND ADDRESS Inveresk Research International Limited, Musselburgh, EH21 7UB, Scotland		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A.3E162720A835.00.104
11. CONTROLLING OFFICE NAME AND ADDRESS Jesse J. Barkley, Jr., U.S. Army Medical Research and Development Command, Fort Detrick, Maryland, U.S.A.		12. REPORT DATE March 1986
		13. NUMBER OF PAGES 70
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Non-classified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

DISTRIBUTION STATEMENT A
Approved for public release
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Principal Investigator: A.B. Wilson

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

HMX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, rats, mice, toxicokinetics, metabolism

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

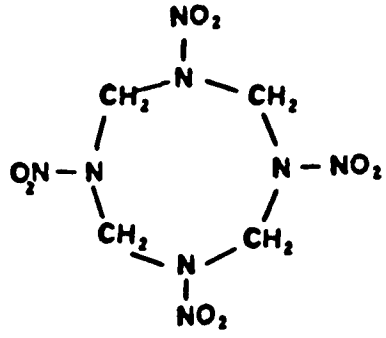
See overleaf.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Non-classified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Abstract



After an oral dose of 500 mg/kg^{-1} to rats, 85% was eliminated in the faeces in 4 days. The equivalent figure for mice was 70%. Plasma and expired CO_2 levels were very low.

Intravenous administration to rats resulted in 61% being eliminated via the urine in 4 days. Peak plasma levels were achieved in 1 h and persisted for 6 h. There was significant and rapid metabolism to very polar metabolites.

The above implies very poor absorption of HMX after oral dosing. Tissue levels were highest in the liver, kidney and brain.

AD

IRI Report No. 2588

HMX: TOXICOKINETICS OF ^{14}C-HMX FOLLOWING ORAL ADMINISTRATION TO THE RAT AND MOUSE AND INTRAVENOUS ADMINISTRATION TO THE RAT

Final Report by:

B.D. Cameron

March 1986

Supported by:

U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, Maryland, 21701

Contract No. DAMD-17-80-C-0053
IRI Project 415669PK

Inveresk Research International Limited
Musselburgh, EH21 7UB, Scotland

Contracting Officer's Technical Representative:

Jesse J. Barkley, Jr.
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

FOREWORD

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."

A.B. Wilson, B.V.Sc., M.R.C.V.S.,
D.A.B.T.
Principal Investigator

Project No. 415669PK and Amendments 1, 2, 3, 4 and 5 Report No. 2588

QUALITY ASSURANCE AUTHENTICATION

The conduct of this study has been subjected to periodic inspections by the IRI Quality Assurance Unit. The dates of inspection are given below.

IRI Project No. 415669PK

Report No. 2588

Date of Q.A. Inspection	Date of Report to Management
2 October 1980	2 October 1980
8 October 1980	16 October 1980
9 October 1980	16 October 1980
13 May 1981	14 May 1981

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.

Signed: Andrew Waddell (Head of Quality Assurance)

Date: 26th March 1986

CONTENTS

SUMMARY

Oral Administration of ^{14}C-HMX (500 mg.kg^{-1}) to Rats and Mice

Following oral administration of ^{14}C-HMX to the rat, radioactivity was rapidly eliminated mainly in the faeces. Thus during 4 days, 85% was eliminated in the faeces, 4% in the urine, and 0.7% retained in the animal body. During 48 h ca 0.5% was eliminated as $^{14}CO_2$.

Following oral administration of ^{14}C-HMX to the mouse, radioactivity was rapidly eliminated mainly in the faeces. Thus during 4 days, 70% was eliminated in the faeces, 3% in the urine, and 0.6% retained in the animal body. During 48 h ca 1% was eliminated as $^{14}CO_2$.

Following oral administration of ^{14}C-HMX to the rat and mouse, plasma levels of radioactivity were very low indeed and at peak plasma levels (6 h post dose 6-10 μg.ml^{-1}) less than 0.1% of the administered dose was present in the plasma circulation.

Thin layer chromatographic analysis of the radioactivity eliminated in the faeces of rats and mice following oral administration of ^{14}C-HMX, showed that faecal radioactivity was mainly unchanged ^{14}C-HMX.

Intravenous Administration of ^{14}C-HMX (2 mg.kg^{-1}) to Rats

Following intravenous administration of ^{14}C-HMX to the rat, radioactivity was rapidly eliminated mainly in the urine. Thus during 4 days, 61% had been eliminated in the urine, 3% in the faeces and 5% retained in the animal body. During 48 h ca 6% was eliminated as $^{14}CO_2$. A proportion of the administered radioactivity was even more slowly eliminated. During 3-4 days post dose only 0.6% was eliminated in urine and faeces.

Following intravenous administration of ^{14}C-HMX to the rat, plasma levels of radioactivity increased during 1 h post dose and peak levels (1 μg equiv.ml^{-1} male and 0.5 μg equiv.ml^{-1} females) were maintained for 6 h. At 24 h levels of radioactivity had fallen significantly (0.2 μg equiv.ml^{-1}), thereafter levels of radioactivity in plasma fell very slowly.

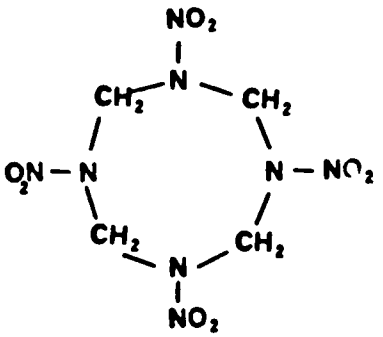
A comparison of urine and plasma levels of radioactivity following intravenous and oral administration of ^{14}C-HMX to rats suggested that <5% of an oral dose of ^{14}C-HMX had been absorbed following the oral administration.

Tissue concentrations of radioactivity following an intravenous dose of ^{14}C-HMX were generally higher than plasma. Highest concentrations were observed in liver and kidney and lowest levels in brain.

Examination of the nature of metabolites eliminated in urine and faeces and retained in tissues and plasma showed significant and rapid metabolism of ^{14}C-HMX to very polar components.

INTRODUCTION

As part of the biological safety evaluation of HMX (a high explosive), the toxicity of the substance has been investigated in rats, mice and rabbits (IRI Project No. 415669).



The present study investigates the metabolism and pharmacokinetics of ^{14}C-HMX in the rat and the mouse.

Investigations in the first instance were limited to studies following oral administration of ^{14}C-HMX. Results of these investigations suggested that ^{14}C-HMX was poorly absorbed by the oral route, the studies were therefore extended to include examination of the pharmacokinetics of ^{14}C-HMX in the rat following the intravenous route of administration.

The studies were performed according to the following protocols:

415669PK: The pharmacokinetics of ^{14}C-HMX following oral administration to the rat and mouse.

415669PK First Amendment:	The pharmacokinetics of ^{14}C-HMX following intravenous administration to the rat.
415669PK Second Amendment:	Not performed.
415669PK Third Amendment:	The pharmacokinetics of ^{14}C-HMX following intravenous administration to the rat. Analysis of individual tissues.
415669PK Fourth Amendment:	Further investigation of urinary ^{14}C-HMX metabolites.
415669PK Fifth Amendment:	Investigation of the nature of radioactivity in rat tissues following a single intravenous dose of ^{14}C-HMX.

These studies were performed at the following locations:

Elphinstone Research Centre, field station of Inveresk Research International and Inveresk Gate, Musselburgh.

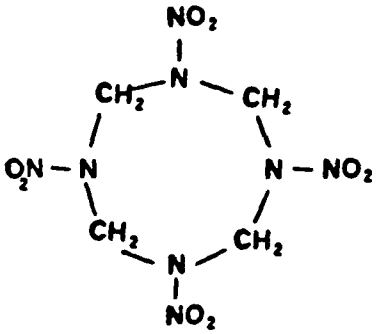
Time of initiation: 17 September 1980
Time of completion: 7 January 1983

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited.

EXPERIMENTAL PROCEDURES

Materials

Carbon-14 labelled HMX, (93.3 mg, Batch No. 1221-068, 5.93 mCi.m mole^{-1}) was received from New England Nuclear on 29 August 1980. The compound was received in the form of a white crystalline solid and was stored deep frozen. The structure of ^{14}C-HMX is shown below.



Non-radioactive HMX and RDX were received from the Royal Ordnance Factory as white suspensions containing approximately 20% (w/w) water for the preparation of analytical standards and for the dilution of the ^{14}C-HMX. Both HMX and RDX were dried to a constant weight in a water-heated oven before use.

"Unisolve" liquid scintillation fluid was obtained from Koch-Light Laboratories Limited, Colnbrook, U.K.

Dimethylsulphoxide (DMSO) used in intravenous dosing, was supplied by BDH Chemicals Ltd., Poole, Dorset, U.K.

Carbosorb^{R} CO_2 absorbing solution and Permafluor V^{R} scintillation fluid were used in conjunction with the Packard Tri-Carb 306 sample oxidiser, and were supplied by Packard Instrument Company Inc., Illinois, U.S.A.

Standards containing a known amount of carbon-14, used to estimate efficiencies of combustion, were obtained from the Radiochemical Centre, Amersham, U.K.

Hypersil, Hypersil ODS (5 µm diameter), Partasil and Co-Pell ODS (20 µm diameter), packing materials for HPLC, were obtained from Shandon Southern Products Limited, Runcorn, Cheshire.

Mobile phase solvents for HPLC were of HPLC grade.

All other reagents used were of analytical reagent grade.

Pre-coated silica gel TLC plates (60 F_{254}, layer thickness 0.25 mm) were supplied by E. Merck, Darmstadt, Germany.

X-ray film (Kodirex^R), developer (DX-80) and fixer (FX-40) were obtained from Kodak Limited, U.K.

Specific Activity and Radiochemical Purity of ^{14}C-HMX

^{14}C-HMX (93.3 mg) was received from New England Nuclear at a stated specific activity of 5.93 mCi.mmol^{-1} (Appendix 1).

Two batches of ^{14}C-HMX were prepared, one of low specific activity for oral administration and one of high specific activity for intravenous administration.

For the low specific activity material ^{14}C-HMX (49.9 mg) was diluted with HMX (9.86 g) in acetonitrile to yield 9.79 g ^{14}C-HMX of specific activity 0.905 μCi.mg^{-1} (26.80 μCi.mmol^{-1}).

For the high specific activity material ^{14}C-HMX was used undiluted. The specific activity was measured at IRI and found to be 17.65 μCi.mg^{-1} (5.22 mCi.mmol^{-1}) and this was the figure used throughout.

Specific activities were determined by U-V spectrophotometry at 225 nm and scintillation counting.

The radiochemical purity of both batches of the prepared ^{14}C-HMX were estimated using TLC in 2 of the following solvent systems.

a) Dichloromethane:acetonitrile (80:30, v/v)
b) Petroleum ether (60-80°C):acetone (60:36, v/v)
c) Petroleum ether (40-60°C):acetone:acetonitrile (60:35:20, v/v/v)

The radiochemical purity of the high specific activity material was assessed as 99.0% and 99.0% pure in solvent systems a and b respectively. The radiochemical purity of the low specific activity material was assessed as 98.3% and 99.4% pure in solvent systems a and c respectively.

Animals and Husbandry

Animal Receipt

For studies involving the oral administration of ^{14}C-HMX to rats, 12♂ and 12♀ Fischer 344 rats were received from Charles River U.K. Limited on 30 September 1980. Mean body weights on arrival were 142 g (♂) and 114 g (♀). A further 5♂ and 5♀ Fischer 344 rats were received from Charles River, U.K. Limited, on Wednesday 22 October 1980. Mean body weights on arrival were 150 g (♂) and 138 g (♀).

For studies involving the oral administration of ^{14}C-HMX to mice, 32♂ and 32♀ B6C3F mice were received from Charles River U.K. Limited, on 7 October 1980. Mean body weights on arrival were 21 g (♂) and 18 g (♂).

For studies involving the intravenous administration of ^{14}C-HMX to rats, 21♂ and 19♀ Fischer 344 rats were received from Charles River U.K. Limited, on 7 May 1981. Mean body weights on arrival were 122 g (♂) and 120 g (♀).

Husbandry

All animals were received at Elphinstone Research Centre, field station of Inveresk Research International Limited. They were uniquely earmarked on arrival and were allowed one week acclimatisation period before dosing. Quarantine restrictions were maintained throughout all animal studies.

After dosing, those animals used in plasma level studies were housed individually in polycarbonate cages with raised mesh floors to inhibit coprophagy. Animals used in balanced excretion studies were housed individually in glass metabowls specially designed for the separate collection of urine and faeces. Except for an 18 h pre-dose fast animals were fed on BP Nutrition Rat and Mouse No. 1 Diet. Water was available ad libitum.

Mean environmental conditions were as follows:-

Rat oral dose studies: Mean temperature was 21°C (range 19-23°C); mean humidity was 55% (range 50-58%).

Mouse oral dose studies: Mean temperature was 19°C (range 17-21.5°C); mean humidity was 51% (range 46-57%).

Rat intravenous dose studies: Mean temperature was 22°C (range 18-24°C); mean humidity was 48% (range 40-55%).

Animal house records are retained in the project archives.

Dose Preparation and Procedure

Oral Administration to Rats

The target dose level for each animal was 500 mg.kg^{-1} body weight. Two stock suspensions of ^{14}C-HMX in 0.1% carboxy methyl cellulose (CMC) solution were prepared at concentrations of ca 23.83 mg.ml^{-1} and 250.02 mg.ml^{-1}. The homogeneity of each suspension was maintained throughout the dosing period by use of a magnetic stirrer. Each animal received a target of 3 ml of the respective dosing suspension by oral gavage.

In order to determine the dose delivered to each animal several mock doses of 3 ml of dose suspension were dispensed throughout the dosing period. These 'mock' doses were subsequently adjusted to a volume of 100 ml with acetonitrile and aliquots were removed for radioactivity determination. The actual doses received by each animal are given in Appendix 2.

Oral Administration to Mice

The target dose level for each animal was 500 mg.kg^{-1} body weight. A stock suspension of ^{14}C-HMX in a 1% CMC solution was prepared at a concentration of ca 23.01 mg.ml^{-1} and individual doses were dispensed from this suspension. Due to a significant difference between male and female mean body weights, the dose volume was different for each sex, with the males receiving 0.5 ml and the females receiving 0.42 ml.

Mock doses of both the male and female target dose volumes were dispensed throughout the dose period for dose determination as above.

The homogeneity of the dose suspension was maintained throughout the dosing period by use of a magnetic stirrer. The actual doses received by each animal are given in Appendix 3.

Intravenous Administration to Rats

The target dose level for each animal was 2 mg.kg^{-1} body weight. As a result of different dosing dates, 3 separate dose solutions of ^{14}C-HMX in dimethylsulphoxide (DMSO) were prepared. Each rat received 30 µl of the respective dosing solution directly into the saphenous vein via a 100 µl Hamilton syringe. In order to determine the radioactivity received by each animal, mock doses were taken throughout the dosing period. Each mock dose was subsequently adjusted to 10 ml with DMSO, and aliquots were removed for radioactivity determination.

This value was used to calculate the dose received by each animal.

The actual doses received by each animal are recorded in Appendix 4.

Note:- Particular difficulties were associated with the intravenous administration of ^{14}C-HMX. HMX is virtually insoluble in all biologicaly compatible solvents. It was decided therefore to utilise the miniumum volume of dimethylsulphoxide (DMSO). The normal procedure of withdrawal of blood into the needle and syringe could not be allowed because any presence of aqueous media precipitated ^{14}C-HMX. The small volume (30 µl) was injected directly into the vein and assessment of success was made following the administration as to whether any of the dose was extravascular.

Animal Experimentation

Oral Administration to Rats

Ten of the male rats and 10 of the female rats each received a single oral dose of ^{14}C-HMX. Following administration, 10 rats (5♂ and 5♀ were each placed singly in all glass metabolism cages. In addition, expired CO_2 was collected independently from 1♂ and 1♀ for a period of 48 h post dose. Urine for the periods 0-6, 6-24, 24-48, 48-72 and 72-96 h and faeces for the periods 0-24, 24-48, 48-72 and 72-96 h post dose, were collected and the radioactivity determined. For the time periods up to 24 h, urine and faeces was collected deep frozen in solid CO_2 cooled containers. At the end of the collection periods, the animals were sacrificed and the radioactivity in the carcass and gastro-intestinal tract was subsequently determined.

Blood samples from the remaining 10 rats (5♂ and 5♀) were taken from the tail vein the following times post dose: 0.25, 0.5, 1, 2, 4, 6, 24, 48 and 72 h post dose. Total radioactivity was subsequently determined in the plasma of each blood sample.

Subsequent to the above a further 6 male and 6 female rats were each given a single oral dose of ^{14}C-HMX. Two male rats and two female rats were sacrificed at 12, 18 and 24 h post dose by chloroform inhalation. Blood was removed via the inferior vena cava into heparinised tubes at sacrifice. Total radioactivity was subsequently determined in the presence of each sample.

A summary of the above procedures is outlined in Appendix 2.

Oral Administration to Mice

Following administration to 10 mice (5♂ and 5♀), each animal was placed individually in all glass metabolism cages. Expired CO_2 was collected from one male and one female animal for a period of 48 h post dose. Urine for the periods 0-6, 6-24, 24-48, 48-72 and 72-96 h and faeces for the periods 0-24, 24-48, 48-72 and 72-96 post dose were collected and the radioactivity determined. For the time periods up to 24 h, urine and faeces were collected deep frozen in solid CO_2 cooled containers. At the end of the collection periods the animals were sacrificed and the radio-activity in the carcass and gastro-intestinal tract was determined. A further group of 48 mice (24♂ and 24♀) were each given a single oral dose of ^{14}C-HMX as described previously. Six animals (3♂ and 3♀) were sacrificed at each of the following times post dose: 0.5, 1, 2, 4, 8, 24, 48 and 72 h. Blood was removed from the inferior vena cava and total radioactivity was determined in the plasma.

A summary outlining the above procedures is recorded as Appendix 3.

Intravenous Administration to Rats

Ten rats (5♂ and 5♀) were each given a single intravenous dose of ^{14}C-HMX and placed independently in all glass metabolism cages. Urine for the periods 0-6, 6-24, 24-48, 48-72 and 72-96 h and faeces for the periods 0-24, 24-48, 48-72 and 72-96 h post dose were collected and the radioactivity determined. For the time periods up to 24 h, urine and faeces were collected deep frozen in solid CO_2 cooled incubators. In addition, expired CO_2 was collected for 48 h from 2 animals (1♂ and 1♀). At the end of excreta collection the 5♂ and 5♀ rats were sacrificed. In 3♂ and 3♀ rats radioactivity was separately determined in the carcass and gastro-intestinal tract. In the remaining 4 rats the levels of radioactivity in the following tissues were determined: gastro-intestinal tract, whole blood, plasma, brain, lung, heart, liver, kidney, thymus, spleen, fat, skeletal muscle, bone, testes/seminal vesicles, ovaries/uterus and remaining carcass.

A further 10 rats (5♂ and 5♀) each received a single intravenous dose of ^{14}C-HMX. Blood was removed from the tail veins of these animals at the following times post dose: 2, 7, 15, 30 min, 1, 2, 4, 6, 24, 48 and 72 h. Total radioactivity was determined in the plasma.

Eight rats (4♂ and 4♀) each received a single intravenous dose of ^{14}C-HMX. Four rats (2♂ and 2♀) were each sacrificed at 2 min and 24 h post dose. Blood was removed from the inferior vena cava for radioactivity determination. In addition the level of radioactivity in the following tissues was determined: gastro-intestinal tract, whole blood, plasma, brain, lung, heart, liver, kidney, thymus, spleen, fat, skeletal muscle, bone, testes/seminal vesicles, ovaries/uterus, and the remaining carcass.

A summary outlining the above procedures is recorded as Appendix 4.

Storage of Samples

All biological samples were stored in individual uniquely identifiable containers, and all samples except plasma were stored deep frozen (-20°) until taken for analysis. Plasma samples were stored at 4°C for the minimum time prior to sampling. After sampling, plasma samples were stored deep frozen.

Quantitation of Radioactivity

Quantitation was analysed using a liquid scintillation analyser (Philips, Holland), with automatic quench correction by external standard-channels ratio. Each individual sample was counted for 10 min or for the time taken to detect 900,000 counts. Where possible, samples were measured at least in duplicate. Vials were allowed to heat and light stabilise overnight prior to analyses. Prior to calculation of each result, a background count rate was determined and subtracted from each sample count rate. A limit of

reliable determination of 30 dpm above the background count rate has been instituted in these laboratories. On statistical grounds, errors associated with the mean of duplicate determinations above this value are less than 12% (CV). Where results have arisen from data below the limit of reliable determination the fact is so noted. Similarly, when a result has arisen from data less than 10 dpm above background, the error is such that the individual result is outside the 95% confidence limit (i.e. the result is not significantly different from background). Data resulting from estimations of less than 10 dpm above background are identified.

Samples were prepared for analysis as follows:

Liquid Samples

Aliquots of urine, plasma, dose determination and residue solutions were made up to 1.0 ml or 4.0 ml with distilled water if necessary and mixed with "Unisolve" scintillator (10 ml).

Whole Blood

Whole blood samples (ca 0.2 g-0.5 g) were combusted using a Packard Tri-carb 306 automatic sample oxidiser. The resultant $^{14}CO_2$ was collected by absorption in Carbosorb^R, to which Permafluor V^R scintillation fluid was added. Combustion of standards showed recovery efficiencies to be in excess of 94% throughout.

Solid Samples

Whole carcasses of rats and mice were finely chopped prior to homogenisation with the addition of CMC (carboxy methyl cellulose) as a stabiliser.

Faeces and gastro-intestinal tract samples were homogenised in water with CMC added. Two samples were removed from each, and each subsample was analysed in duplicate. Aliquots of homogenate (ca 0.5 g) were combusted as described for "Whole Blood" above.

Organs and tissues were finely chopped. Duplicate aliquots (ca 0.5 g) were taken from each, and combusted as described above.

TLC Absorbent

Bands of absorbent corresponding to radioactive areas on TLC plates were removed into scintillation vials containing distilled water (4 ml). The resulting mixture was subjected to ulstrasonication to disperse the solid phase and "Unisolve" (10 ml) was added to produce a thixotropic gel suitable for scintillation counting.

Examination of the Nature of Radioactivity in Urine, Faeces, Plasma and Tissue Sample

Urine and faeces obtained from the oral and intravenous dosing studies in the mouse and rat for the time periods 0-6, 6-24, 24-48, 48-72 and 72-96 h were pooled separately for male and female animals. Unchanged test substance was determined in the animals. Additionally the pattern of metabolites was determined in those of the above samples that contained the highest proportion of radioactivity.

Plasma samples for unchanged test substance analysis were obtained from 8 animals (4♂ and 4♀) at the peak plasma level (for oral dosed animals) or 2 min (for intravenous dosed animals) and 24 h.

Selected urine samples were subjected to enzymatic and chemical deconjugation procedures.

RESULTS

The Pharmacokinetics of Radioactivity Following Oral Administration of ^{14}C-HMX (500 mg.kg^{-1}) to the Rat and Mouse

Rates and Routes of Excretion of Total Radioactivity

Following single oral administration of ^{14}C-HMX (500 mg.kg^{-1}) to the rat, radioactivity was rapidly and significantly eliminated mainly in the faeces. Thus after 96 h, 85% had been eliminated in the faeces, 4% in the urine and only 0.7% retained in the gastro-intestinal tract and carcass. (Table 1, Figure 1). In 2 animals, $^{14}CO_2$ was collected during the first 48 h post dose. In these 2 animals a mean of 0.5% was eliminated as $^{14}CO_2$ (Table 3). There appeared to be no difference in the results obtained from male or female rats.

Following single oral administration of ^{14}C-HMX (500 mg.kg^{-1}) to the mouse, radioactivity was rapidly eliminated in the faeces with only a small proportion eliminated in the urine. Thus after 96 h, 70% had been eliminated in the faeces, 3% in the urine and only 0.6% retained in the gastro-intestinal tract and carcass (Table 2, Figure 1). Similarily in the 2 animals where $^{14}CO_2$ was collected during the first 48 h post dose a mean of 1.1% was collected as $^{14}CO_2$.

Thus in the rat and the mouse, radioactivity, following oral administration, was rapidly eliminated in the faeces. Observed levels in the carcass and in the urine, however showed that at least a proportion of the administered dose had been absorbed following oral administration.

Plasma Levels of Total Radioactivity

Following an oral dose of ^{14}C-HMX (500 mg.kg^{-1}) to rats and mice, levels of radioactivity in plasma increased slowly during the first 6 h post dose (Tables 4 and 5, Figure 2). Plasma levels of radioactivity were very low indeed (relative to the dose administered) and for both species peaked between 6 and 12 µg equiv.ml^{-1} (Tables 4 and 5, Figure 2). Assuming a plasma volume of 45 ml.kg^{-1}, plasma concentrations at peak accounted for only 0.07% of the administered dose in the whole plasma circulation.

During 24 h to 72 h post dose, levels of radioactivity fell slowly to (in many cases) below the limit of reliable determination.

A more detailed interpretation of the pharmacokinetics of total radioactivity is difficult because of data variability with observed levels being close to the limit of reliable determination.

Note: During the period 6-24 h post dose, peak levels of radioactivity probably occurred. Additional animals were used to examine plasma concentration during this period to observe whether these were

significantly higher than those observed at 6 h (Table 4b). Levels of radioactivity observed in these limited numbers of animals were within the expected range for kinetic animals at 6 h post dose.

During this phase, therefore, the results suggest that the proportion of ^{14}C-HMX absorbed may be low. This suggestion is supported by the observation of the low levels of radioactivity in urine and plasma. An alternative interpretation, however, would be: significant absorption followed by rapid elimination in the bile. The second alternative seemed unlikely since plasma levels and urine levels were low. It was appropriate however to extend the investigations of the pharmacokinetics of ^{14}C-HMX following a systemic route of administration in one of the species:

The route chosen was intravenous.
The species chosen was the rat.

The dose level chosen was 2 mg.kg^{-1} (as opposed to 500 mg.kg^{-1} oral). This dose level may be considered as representative of the absorbed portion of the dose following oral administration.

Examination of the excretion and plasma pharmacokinetics, following the intravenous dose, would confirm (or otherwise) the poor absorption following oral administration.

The Pharmacokinetics of Radioactivity Following Intravenous Administration of ^{14}C-HMX (2 mg.kg^{-1}) to the Rat.

Rates and Routes of Excretion of Total Radioactivity

Following single intravenous administration of ^{14}C-HMX to the rat, radioactivity was rapidly and significantly eliminated mainly in the urine. Thus after 96 h, 61% had been eliminated in the urine, 3% in the faeces and 5% retained in the gastro-intestinal tract and carcass (Table 6, Figure 3). In 2 animals expired air was trapped for CO_2 during 48 h post dose and approximately 6% of the radioactive dose was eliminated as $^{14}CO_2$ (Tables 6 and 7). There appeared to be no difference in the results obtained from male or female rats.

Thus in the rat, systemically administered radioactivity is rapidly and significantly eliminated in the urine. However, the rate of elimination is rapid only at early time periods. During 72-96 h, only 0.6% was eliminated whilst 5% was retained in the carcass (Table 6).

Recoveries of radioactivity were low (70-80%) $^{14}CO_2$ was collected from only 2 animals during the first 2 days post dose. However, during 2-5 days post dose (outside the period of measurement) elimination of $^{14}CO_2$ may have been significant and other possible volatile metabolites (e.g. $^{14}CH_4$) were not collected. This may have contributed to the low recoveries observed.

Plasma Levels of Total Radioactivity

Following intravenous administration of ^{14}C-HMX (2 mg.kg^{-1}) to 5 male and 5 female rats radioactivity increased during the first hour post dose and generally maintained a plateau of radioactivity for up to 6 h post dose at a concentration of 1 µg equiv.ml^{-1} (males) or 0.5 µg equiv.ml^{-1} (females). See Tables 8 and 9.

During 6-24 h post dose, levels of radioactivity fell significantly and both male and female rats maintained similar levels of radioactivity (0.2 µg equiv.ml^{-1}) at 24 h post dose. Thereafter plasma levels of radioactivity fell more slowly and at 72 h post dose, levels of 0.05 µg equiv.ml^{-1} were circulating in plasma.

The unusual observation of increasing concentration of radioactivity following an intravenous dose can be generally attributed to the rapid formation of a metabolite with a smaller volume of distribution than the parent compound administered.

Assuming a plasma volume of 45 ml.kg^{-1}, at peak plasma concentrations (1-6 h post dose) a mean of ca 4% of the administered radioactive dose was circulating in plasma.

Comment on the Systemic Absorption of an Oral Dose of ^{14}C-HMX Administered to Rats

Estimates of the systemic absorption of radioactivity following an oral dose may be made by comparing the urinary levels of radioactivity following oral and intravenous administration. viz:

$$\text{Proportion absorbed} = \frac{\text{\% dose in urine (p.o. admin)}}{\text{\% dose in urine (i.v. admin)}} \times 100$$

$$= 6\%$$

Alternatively the areas under the plasma profiles following intravenous and oral administration may be compared giving the bioavailability of total radioactivity. However, in the present study, levels of radioactivity in plasma were at or below the limits of reliable determination at later time periods. Thus, comparison of peak or plateau levels may be more appropriate. viz:

$$\text{'Bioavailability' of the oral dose} = \frac{\text{'Peak' oral/oral dose}}{\text{'Peak' intravenous/intravenous dose}} \times 100$$

$$= \frac{8/500}{0.8/2} \times 100$$

$$= 4\%$$

It may be considered that following oral administration of ^{14}C-HMX (500 mg.kg^{-1}) to rats that approximately 5% was absorbed into the systemic circulation.

Tissue Levels of Radioactivity Following Intravencus Administration of ^{14}C-HMX (2 mg.kg^{-1})

Following single intravenous administration of ^{14}C-HMX (2 mg.kg^{-1}) to 6 male and 6 female rats, radioactivity was measured in tissues and organs of separate animals. Tables 10, 11 and 12 outline the results from animals sacrificed at 2 min, 24 h and 96 h post dose respectively.

At 2 min post dose highest levels of radioactivity were observed in lung and heart with lowest levels in the brain. During 24 h post dose levels in tissues fell considerably with major concentrations detected in liver and kidney. During 96 h post dose, levels of radioactivity fell further with maximum levels in kidney and liver, the major organs of metabolism and elimination. At all times post dose levels in the brain were very low indeed. Selected mean tissue concentrations are outlined below and Figure 5.

	Radioactivity (µg equiv.g^{-1})		
	2 min	24 h	96 h
Whole blood	2.15	0.22	0.07
Plasma	1.58	0.18	0.05
Lung	15.39	0.32	0.19
Liver	4.25	0.83	0.26
Kidney	3.96	0.74	0.36
Skeletal muscle	1.28	0.22	0.08
Brain	0.22	0.09	0.04

Total recoveries of radioactivity in the rat body at 2 min, 24 h and 96 h post dose were 90%, 17% and 5% respectively (Table 13).

Tissue to plasma ratios of radioactivity are detailed in Tables 14, 15 and 16 and summarised below.

	Tissue/Plasma Radioactivity Ratios		
	2 min	24 h	96 h
Whole blood	1.5	1.2	1.5
Lung	7.6	1.8	4.3
Liver	2.3	4.6	6.1
Kidney	2.2	4.1	8.1
Skeletal muscle	0.7	1.2	1.7
Brain	0.1	0.5	0.8

Thus the proportionate distribution of radioactivity changed significantly with time. With the particular exception of lung (at 2 min), the tissue to plasma ratios of radioactivity increased with time. In the particular case of lung at 2 min it must be stated that lung tissue is the first tissue to meet a bolus of intravenously administered ^{14}C-HMX. At 2 min post dose ^{14}C-HMX could be out of equilibrium with plasma and other tissues.

Determination of the Level of Unchanged ^{14}C-HMX in Urine, Plasma and Faeces

Thin Layer Chromatography of Urine and Faeces Extracts From Rats and Mice Following ORAL Administration of ^{14}C-HMX (500 mg.kg^{-1})

Male and female 6-24 h rat and mouse urine collection and male and female rat and mouse faeces were separately pooled. Pooled urine samples (2-20 ml) were extracted with 10 volumes of acetonitrile. Pooled faeces samples (ca 3 g) were extracted with 2 x 5 ml acetonitrile. Each urine and faeces extract was reduced to dryness *in vacuo*, recovery of radioactivity was virtually quantitative. Each dry residue was redissolved in a minimal volume of acetonitrile prior to thin layer chromatography in dichloromethane:acetonitrile (80:30 v/v). After development the level of ^{14}C-HMX and major separate components were determined by excision of the appropriate areas of silica gel corresponding to the major areas of radioactivity (detected by apposition autoradiography).

The proportions of each separated component in each extract are detailed in Table 17a.

Examination of the results showed that almost all of the radioactivity eliminated in faeces was excreted as unchanged ^{14}C-HMX. Urine samples contained only a very small proportion of radioactivity compared to faeces thus the qualitative results for urine must be viewed with caution (i.e. the high proportion of ^{14}C-HMX in some urine samples may be caused by transfer of faecal radioactivity to urine during separation and collection).

Table 17b converts the proportion of radioactivity attributable to each component to a percentage of the administered dose.

From the preceding sections it is clear that only a very small proportion of ^{14}C-HMX is absorbed following oral administration of ^{14}C-HMX to rats and mice. ^{14}C-HMX is eliminated for the most part unchanged in the faeces. However a significant (if small) proportion of ^{14}C-HMX is absorbed following oral administration (as evidenced by urine and particularly plasma levels of radioactivity). It is important to describe the fate of this proportion of radioactivity absorbed without the hindrance of low levels of radioactivity and possible contamination of eliminated urine with high levels of unabsorbed radioactivity in the faeces.

Examination of the metabolites following systemic administration of a low dose of ^{14}C-HMX followed, and the disposition or extent of metabolism of ^{14}C-HMX in this situation may be related to the fate of the absorbed ^{14}C-HMX following oral administration.

Thin Layer Chromatography of Urine Extracts from Rats Following INTRAVENOUS Administration of ^{14}C-HMX (2 mg.kg^{-1})

Urine samples (0-6 h, 6-24 h and 24-48 h) were pooled for male and female animals separately. Samples were extracted with 10 volumes of acetonitrile and reduced to dryness by thin film rotory evaporation. Each dried extract was transferred to small vials using a minimum volume of 90% methanol:10% water. Extracts were reduced to dryness under nitrogen and redissolved in a minimum volume of 90% methanol 10% water. Recovery of radioactivity using this technique was almost quantitative (87-98%).

Thin layer chromatography of the above extracts was performed using silica gel TLC plates developed in dichloromethane:acetonitrile (80:30 v/v). Each sample was co-chromatographed with HMX. Areas of silica gel corresponding to high levels of radioactivity were visualised using apposition autoradiography.

Four significant radioactive components were detected, HMX (RF 0.51), two minor metabolites at RF 0.08 and 0.03 (Met 1 and 2 respectively) and material retained at the origin. The areas corresponding to these components were excised and measured for total radioactivity and the proportions corresponding to each calculated (Table 18a). Thus the minor metabolites (1 and 2) accounted for only 1-2% of total radioactivity in both male and female urine with approximately equal proportions of HMX and polar (origin) material making up the remainder. The proportion of radioactivity corresponding to HMX generally decreased with time and the proportion of HMX in the pooled female urine samples analysed contained higher proportions of HMX than corresponding male urine. A quantitative estimate of the amount of each component eliminated is given in Table 18b by reference to the proportion of the administered dose detected in each sample. Thus during 48 h the following amounts of each component had been eliminated.

	Percentage Dose Eliminated (0-48 h urine)	
	Male Rats	Female Rats
HMX	24.5	37.8
Met 1	0.2	0.2
Met 2	1.0	1.0
Origin	28.3	16.7

Later urine samples and faeces samples were not analysed because of the lower levels of radioactivity observed in these samples.

High Performance Liquid Chromatography of Plasma Extracts of Rats Following INTRAVENOUS Administration of ^{14}C-HMX (2 mg.kg^{-1})

Terminal plasma samples collected at 2 min and 24 h following intravenous administration to male and female rats were analysed for ^{14}C-HMX separately.

Each plasma sample was extracted with 2 x 5 volumes of acetonitrile. The acetonitrile extracts of plasma at 2 min post dose contained greater than 90% of the original radioactivity. Extracts of plasma at 24 h post dose contained 15-30% of the total radioactivity (Table 19). Extracts were reduced to dryness under nitrogen and reconstituted in a minimal volume of 90% methanol:10% water and subjected to reverse phase HPLC analysis using the following system.

Column (1)	4 x 0.5 cm Co-Pell ODS
(2)	25 x 0.8 cm Hypersil ODS
Mobile Phase	25% acetonitrile in water at 2 ml.min^{-1}

Control plasma samples containing unchanged ^{14}C-HMX were similarly processed. ^{14}C-HMX was quantitatively extracted.

Eluate was collected at the retention time of authentic ^{14}C-HMX and the radioactivity associated with this fraction compared with the total eluate. Recovery from the column was quantitative. Results are given in Table 19.

Thus at 2 min post dose most of the radioactivity circulating in plasma corresponds to unchanged ^{14}C-HMX (0.7-1.8 µg.ml^{-1}) whereas at 24 h only ca 10% of the circulating radioactivity corresponded to ^{14}C-HMX corresponding to ca 0.02 µg.ml^{-1}.

Following the observation that low recoveries of radioactivity occurred following extraction of 24 h plasma samples, these samples were subjected to further investigation. Extraction with methanol:water (90:10 v/v) did not significantly improve extractability and most of the radioactivity was associated with the precipitated protein fraction. Hydrolysis with 2M NaOH did improve extractability but this treatment also degraded unchanged ^{14}C-HMX. Much of the radioactivity observed in plasma at 24 h post dose may be strongly bound or incorporated into plasma protein.

Analysis of the Nature of Radioactivity in Urine and Faeces Following INTRAVENOUS Administration of ^{14}C-HMX to Rats Using HPLC

Thin layer chromatographic (TLC) analysis had shown previously that radioactivity in rat urine consisted of 2 major radioactive components ^{14}C-HMX and highly polar material. (See page 18.) Low levels of radioactivity in later urine samples and all faeces samples precluded the examination of these samples by TLC. HPLC was used to confirm the results in urine observed previously by TLC and to extend this

examination to faeces extracts and to extracts of urine samples collected at later times following an intravenous administration of ^{14}C-HMX. Previously pooled urine and faeces samples collected following a single administration of ^{14}C-HMX were extracted with 5 volumes of acetonitrile. The extracts were reduced to dryness and taken up in a minimum volume of 90% methanol in water for subsequent HPLC analysis:

Column (1):	4 x 0.5 cm Co-Pell ODS
(2):	25 x 0.8 cm Hypersil ODS (5 μm)
Mobile:	25% acetonitrile in water at 2 ml.min^{-1}
Detection (1):	u.v. absorbance at 280 nm
(2):	Berthold LB 503 HPLC radioactivity detector with 200 μl homogeneous flow cell and Unisolve^{R} scintillator at 5 ml.min^{-1}

Each sample was co-chromatographed with HMX.

Radioactive components detected in the HPLC eluate of each sample were quantitatively collected by fraction collecting and analysed by scintillation counting.

Typically 2 major radioactive components were detected in each sample analysed (Figure 6) although 3 minor components were also detected.

The pattern of metabolites was similar for urine and faeces in all samples analysed (Table 20a). Samples only varied in the proportion of one to another. Thus 2 major components were detected. Unchanged ^{14}C-HMX (at a retention time of 22 min) accounted for between 68% and 11% of the total radioactivity in each sample. The other major component represented highly polar unchanged material which was unresolved from the solvent front, and accounted for between 14% and 82% of the total radioactivity in each sample.

The proportion of radioactivity associated with HMX in each sample generally decreased with time post dose (Table 20a) and samples from female animals generally had higher concentrations of radioactivity than male animals (Table 20a).

The 3 minor components (A, B and C) accounted for only a small proportion (<2%) of each urine extract. However in faeces the proportion of one of these metabolites was significant (MetC, 10%). The levels of radioactivity eliminated in faeces however only accounted for a very small proportion of the administered dose. Proportions of each component were converted to a percentage of the administered dose (Table 20b). Thus a full account of the extent of metabolism and the proportion of each component could be assessed.

Thus during 0-96 h the following amounts of each component were eliminated in urine (and 0-24 h faeces). See also Table 20b.

	% Dose Eliminated	
	Male Rats	Female Rats
HMX	25.1	40.7
Met A	0.4	0.3
Met B	0.4	0.2
Met C	0.7	1.0
Polar material	29.4	20.1

The polar material however was unresolved from the solvent front and maybe equated with the polar material unresolved from the origin during TLC analysis (See page 18).

Further attempts were made to separate and examine the nature of the polar material by further HPLC analysis and by hydrolysis. See following section.

Examination of the Nature of the Polar Unknown Metabolite of ^{14}C-HMX Present in Urine and Faeces of Rats Following a Single Intravenous Dose of ^{14}C-HMX

It has previously been shown that the main metabolite of ^{14}C-HMX in rats following a single intravenous dose is a polar compound. This material was essentially unretained in the HPLC system used for investigation. This section details the results of further investigations by HPLC to separate or further characterise this component.

The HPLC column system was that used previously:

Column (1): 4 x 0.5 cm Co-Pell ODS
(2): 25 x 0.4 cm Hypersil ODS (5 µm)

Experimental mobile phases varied from (A) water at pH 2.5 with acetonitrile up to 67%, to (B) mixtures of between 4 and 67% acetonitrile (v/v) in water at pH 2.5 in each case with the addition of sodium lauryl sulphate (SLS) at 0.4% (w/v) as an ion-pair reagent. The flow rate was 1.3 ml.min^{-1}.

Radioactive components in the HPLC eluate were detected using either a Berthold LB 503 radioactivity detector with a 200 µl homogeneous flow cell and simultaneously pumped scintillation fluid or eluate collected as fractions for subsequent scintillation counting.

Figures 7-9 show typical radio-HPLC profiles of male rat urine (6-24 h) using a reversed phase ion-pair HPLC system. Figure 10 depicts a non-ion paired reversed phase radio-HPLC profile of male rat urine (24-48 h).

The polar material was found to chromatograph consistently at or close to the solvent front confirming the high polarity of the material. No system investigated yielded adequate retention of the metabolite.

In order to investigate the possibility that the highly polar metabolite is a conjugate, an aliquot of urine was incubated in an equal volume of 2M hydrochloric acid for 1 h at 100°C. At the end of incubation the samples were allowed to cool before being neutralised, frozen, dried in a desiccator and reconstituted in methanol for HPLC as follows:

Column (1):	4 x 0.5 cm Co-Pell ODS
(2):	25 x 0.8 cm Hypersil ODS
Mobile:	20% acetonitrile in water at pH 2.5 containing SLS at 0.4%

Detection of radioactivity in the HPLC eluate was by fraction collection followed by liquid scintillation counting.

On both a qualitative and quantitative basis no real difference was noted in the metabolic pattern of urine as a result of the acid hydrolysis (Figure 11).

Examination of the Nature of Radioactivity Detected in the Tissue of Rats Following a Single Intravenous Dose of ^{14}C-HMX

Selected tissues from male and female rats sacrificed at 2 min, 24 h and 96 h post dose were pooled on the basis of weight and extracted by homogenisation in 5 volumes of methanol. The homogenates were centrifuged and the pellets were re-extracted with a further 5 volumes of methanol. The combined methanol extracts were reduced in vacuo and redissolved in a small volume of methanol for HPLC analysis as follows:

Column (1):	4 x 0.5 cm Co-Pell ODS
(2):	25 x 0.8 cm Hypersil ODS
Mobile:	20% acetonitrile in water at pH 2.5 containing SLS at 0.4%

Detection of radioactivity in the sample eluates was by use of the radioactivity monitor and by fraction collection as described previously.

The metabolic profile of each sample, with the exception of the 2 min liver extract, contained only HMX and/or the polar component (Table 21). The polar metabolite had similar characteristics to the metabolite previously detected in urine and faeces. In the 2 min liver extracts, a further minor component was observed (Figures 12 and 13) with retention characteristics close to that of HMX. This pattern was confirmed when radiodetection was conducted using fraction collection. This component was not observed in other samples analysed.

At 2 min, radioactivity was well extracted from tissue using the above techniques (Table 21) and at this time almost all the radioactivity was present as unchanged ^{14}C-HMX. In liver, however, even at 2 min post dose, a significant proportion of radioactivity extracted (ca 30% see Table 21) was present as the polar component.

At later time periods levels of radioactivity in the tissue were much lower and as such only a limited number of the highest level tissues could be examined. In general, however, the extractability of radioactivity from tissue at later time periods was lower and at later time periods there was a much higher proportion of the polar component (Table 21)

DISCUSSION

Following oral administration of ^{14}C-HMX (500 mg.kg^{-1}) to rats and mice, radioactivity was poorly absorbed into the systemic circulation. Levels of radioactivity observed in plasma were very low indeed and much of the administered dose was eliminated as unchanged ^{14}C-HMX in the faeces. A small but significant proportion (<5%) of an oral dose had been absorbed and therefore the disposition of the absorbed material may be important. Studies in the rat were extended to include intravenous administration of a low dose (2 mg.kg^{-1}) of ^{14}C-HMX. The disposition of ^{14}C-HMX following this route of administration may be considered representative of the systemically absorbed portion of ^{14}C-HMX which was observed following oral administration.

Following intravenous administration of ^{14}C-HMX to rats, radioactivity was rapidly distributed to the tissues. Tissue concentrations, however, fell rapidly with time and radioactivity was rapidly eliminated in the urine. Highest concentrations of radioactivity were associated with the organs of metabolism and elimination (liver and kidney), and no unusual sites of accumulation or retention were identified. It was interesting to note, however, that the lowest levels of radioactivity were observed in brain tissue, suggesting poor transfer of ^{14}C-HMX or its metabolites across blood/brain barrier.

Examination of the nature of radioactivity in tissue and urine samples showed that ^{14}C-HMX was metabolised to very polar components and that the proportion of metabolites *increased* with time. This suggests that there was a more rapid elimination of unchanged ^{14}C-HMX than the metabolites. It is clear, however, that since a proportion of ^{14}C-HMX was metabolised to $^{14}CO_2$ that ring cleavage of ^{14}C-HMX had occurred with further metabolic degradation to intermediary products. These products may be further degraded to $^{14}CO_2$ or indeed be incorporated into body tissues as natural products.

TABLE 1

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Cumulative Excretion of Total Radioactivity from 10 Rats for the Period 0-96 h Following a Single Oral Dose of ^{14}C-HMX at 500 mg.kg^{-1}
(Results Expressed as % of Dose)

Time (h)	1♂	2♂	3♂	4♂	6♂	13♀	14♀	15♀	16♀	21♀	Mean
Urine											
0- 6	0.64	0.78	1.08	0.57	0.83	0.56	0.42	0.53	0.55	0.52	0.65
0-24	2.63	1.79	3.10	1.75	2.46	2.10	1.42	2.06	2.31	1.73	2.14
0-48	3.15	2.17	4.21	3.39	2.97	3.23	2.74	2.61	4.31	2.56	3.13
0-72	3.25	2.43	4.45	3.73	3.10	3.62	3.31	2.81	5.43	2.97	3.51
0-96	3.29	2.55	4.51	3.81	3.15	3.77	3.48	2.88	5.85	3.08	3.64
Faeces											
0-24	77.32	73.33	53.79	65.95	72.77	58.85	64.97	47.35	65.46	55.67	63.56
0-48	85.50	82.06	76.85	80.38	85.31	80.13	84.79	77.84	95.00	70.49	81.84
0-72	85.61	83.15	77.81	82.01	85.44	81.84	86.00	90.58	97.92	71.05	84.15
0-96	85.63	83.31	77.92	83.42	85.47	81.89	89.57	90.60	98.18	71.09	84.72
Cage Wash	0.27	1.46	0.44	1.77	0.81	1.68	0.96	0.22	0.72	1.68	1.00
GI Tract	0.06	0.07	0.09	0.06	0.06	0.06	0.05	0.03	0.12	0.07	0.07
Remaining Carcass	0.05	0.83	0.88	0.67	0.57	0.44	0.60	0.43	0.61	0.42	0.55
Total											
0-6	0.64	0.78	1.08	0.57	0.83	0.56	0.42	0.53	0.55	0.52	0.65
0-24	79.95	75.12	56.89	67.70	75.23	60.95	66.39	49.41	67.77	57.40	65.69
0-48	88.65	84.23	81.06	83.77	88.28	83.36	87.53	80.45	99.31	73.05	84.97
0-72	88.86	85.58	82.26	85.74	88.54	85.46	89.31	93.39	103.35	74.02	87.65
0-96	88.92	85.96	82.43	87.23	88.62	85.66	93.05	93.48	104.03	74.17	88.35
Total Recovery	89.30	88.22	83.84	89.73	90.06	87.84	94.66	94.16	105.48	76.34	89.98

TABLE 2

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Cumulative Excretion of Total Radioactivity from 10 Mice for the Period 0-96 h Following a Single Oral Dose of ^{14}C-HMX at 500 mg.kg^{-1}
(Results Expressed as % of Dose)

Time (h)	1♂	2♂	3♂	31♂	5♂	33♀	34♀	35♀	36♀	37♀	Mean
Urine											
0-6	0.15	0.46	0.36	0.26	0.38	0.16	0.41	0.68	0.62	0.50	0.41
0-24	1.02	1.42	0.99	0.64	0.87	2.40	2.63	2.22	1.24	2.09	1.65
0-48	3.02	2.05	1.42	0.70	1.59	3.76	2.74	3.58	2.60	3.77	2.73
0-72	3.61	2.52	2.49	0.93	1.70	4.01	2.95	3.97	3.17	4.12	3.17
0-96	4.20	2.58	2.88	+	1.78	4.21	2.99	4.06	3.24	4.31	3.36
Faeces											
0-24	26.95	71.47	47.39	54.38	62.84	86.18	81.12	79.37	39.29	46.66	60.14
0-48	39.81	74.00	51.84	71.82	70.65	90.46	88.96	81.57	50.97	54.83	67.49
0-72	53.51	76.39	53.68	72.53	72.64	90.74	89.06	81.68	51.26	55.34	69.68
0-96	57.55	77.04	55.19	+	72.76	90.91	89.13	81.74	55.49	55.83	70.18
Cage Wash	6.53	3.23	3.37	4.47	2.94	1.57	2.62	0.54	5.62	2.34	3.20
GI Tract	0.19	0.24	0.16	3.43	0.48	0.17	0.07*	0.07	0.04*	0.04**	0.16
Remaining Carcass	0.83	0.36*	0.51*	1.74	0.39*	0.47*	0.13**	0.21**	0.32*	0.29**	0.39
Total Excreted											
0-6	0.15	0.46	0.36	0.26	0.38	0.16	0.41	0.68	0.62	0.50	0.41
0-24	28.01	72.89	48.38	55.02	63.71	88.58	83.75	81.59	40.53	48.75	61.80
0-48	42.87	76.07	53.26	72.52	72.24	94.22	91.70	85.15	53.37	58.70	70.01
0-72	57.16	78.93	56.17	73.46	74.34	94.75	92.01	85.65	54.43	59.46	72.54
0-96	61.79	79.64	58.07	+	74.54	95.12	95.12	92.12	85.80	60.14	73.59
Total Recovery	69.30	83.45	62.11	83.10	78.35	97.33	94.94	86.62	61.06	62.47	77.29

+ = Mouse 31♂ was found dead in its cage at end of 72 h period (excluded from mean)
* = Data derived from dpm less than 30 above background
** = Data derived from dpm less than 10 above background

TABLE 3

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Cumulative Excretion of Total Radioactivity Following a Single Oral Dose of ^{14}C-HMX to Rats and Mice (500 mg.kg^{-1})
(Results Expressed as Mean ± S.D.% dose recovered)
Mean ± S.D. of 5♂ and 5♀ rats
Mean ± S.D. of 4♂ and 5♀ mice

Time (h)	Rat	Mouse+
Urine		
0- 6	0.65 ± 0.20	0.41 ± 0.18
0-24	2.14 ± 0.50	1.65 ± 0.68
0-48	3.13 ± 0.69	2.73 ± 0.90
0-72	3.51 ± 0.87	3.17 ± 0.83
0-96	3.64 ± 0.95	3.36 ± 0.89
Faeces		
0-24	63.56 ± 9.58	60.14 ± 20.93
0-48	81.84 ± 6.53	67.49 ± 18.30
0-72	84.15 ± 7.17	69.68 ± 16.14
0-96	84.72 ± 7.35	70.18 ± 15.42
Cage Wash	1.00 ± 0.61	3.20 ± 1.87
GI Tract	0.07 ± 0.02	0.16 ± 0.14
Remaining Carcass	0.55 ± 0.16	0.39 ± 0.20
Total Excreted		
0- 6	0.65 ± 0.20	0.41 ± 0.18
0-24	65.69 ± 9.58	61.80 ± 21.39
0-48	84.97 ± 6.82	69.76 ± 18.51
0-72	87.65 ± 7.54	72.54 ± 16.17
0-96+	88.35 ± 7.79	73.59 ± 15.40
Total*	89.98 ± 7.54	77.29 ± 14.17
†$^{14}CO_2$		
0-48	0.49	1.11

* = Excluding $^{14}CO_2$ which was only measured for 2 animals of each species

† = Mean of 1♂ and 1♀ animal

+ = Mouse 31♂ died between 48 and 72 h post dose and is excluded from the mean

TABLE 4

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Plasma Levels of Total Radioactivity Following a Single Oral Dose of ^{14}C-HMX to Rats at 500 mg.kg^{-1}
(Results Expressed as µg Equiv.ml^{-1} Normalised to a Dose of 500 mg.kg^{-1})

A. Kinetic Samples

	Animal No.										♂	♀
Time (h)	11♂	7♂	8♂	9♂	12A♂	24♀	19♀	20♀	22♀	23♀	Mean	Mean
0.25	0.00*	1.39**	1.02**	1.50**	0.43**	1.12*	0.47*	0.84**	1.26**	1.19**	0.87 ± 0.64	0.98 ± 0.32
0.5	1.00*	0.58**	2.04**	0.98**	2.07**	1.16*	1.82*	0.17*	1.96*	1.74**	1.33 ± 0.68	1.37 ± 0.74
1.0	1.74*	2.67*	3.05*	3.42*	2.23*	1.57**	1.38*	1.81*	2.72*	2.27*	2.62 ± 0.66	1.95 ± 0.54
2.0	5.77*	5.03	5.01*	3.99*	5.12	2.58	2.61*	3.25	3.86	3.19	4.98 ± 0.64	3.10 ± 0.53
4.0	7.75	6.94	4.79	7.01	8.15	3.85*	4.69	4.34*	4.05*	4.00*	6.93 ± 1.30	4.19 ± 0.33
6.0	8.66	8.38	8.59	8.58	8.78	4.92	11.05	5.08	4.92	5.23	8.60 ± 0.15	6.24 ± 2.69
24.0	7.16	8.80	4.87	8.38	7.80	8.07	10.15	8.10	8.92	7.93	7.40 ± 1.54	8.60 ± 0.87
48.0	4.12	5.97	8.14	3.73	3.43	4.75	5.23	4.44	4.11	4.57	3.88 ± 1.39	4.62 ± 0.41
72.0	2.43	3.28	1.41	2.00	2.18	3.22	3.29	2.69	2.40	2.83	2.26 ± 0.68	2.89 ± 0.37

* = Calculated from data less than 30 dpm above background ** = Calculated from data less than 10 dpm above background

TABLE 4 (continued)

B. Single Terminal Samples

Terminal Plasma Levels						
Time (h)	♂		♀		♂ Mean	♀ Mean
12	9.59	11.27	5.46	4.92	10.43	5.19
18	9.14	13.27	5.86	7.07	11.21	6.47
24	7.17	5.07	6.11	8.12	8.66	7.12

TABLE 5

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Plasma Levels of Total Radioactivity Following a Single Oral Dose of ^{14}C-HMX to 48 Mice at 500 mg.kg^{-1}
(Results Expressed as µg Equiv.ml^{-1} Normalised to a Dose of 500 mg.kg^{-1})

Time (h)	Male			Female			Mean ♂ ± S.D.	Mean ♀ ± S.D.
0.5	3.34	2.91	1.32*	1.00*	1.67*	1.28*	2.52 ± 1.06	1.32 ± 0.34
1.0	2.22*	2.69*	2.26*	5.87	2.13*	1.81*	2.39 ± 0.26	3.27 ± 2.26
2.0	2.46*	3.15	2.41*	4.30	3.53	3.19	2.67 ± 0.41	3.67 ± 0.57
4.0	3.61*	3.17	3.53	5.03	5.34	5.07	3.44 ± 0.23	5.15 ± 0.17
8.0	4.22	3.57	4.49	7.62	5.45	5.63	4.09 ± 0.47	6.23 ± 1.20
24.0	5.68	4.49	3.78	7.26	7.82	4.47	4.65 ± 0.96	6.52 ± 1.79
48.0	7.40	5.70	-	6.08	4.22	5.46	•• 6.55 ± 1.20	5.25 ± 0.95
72.0	0.45**	1.47*	1.52*	5.00	0.72**	2.89	1.15 ± 0.60	2.87 ± 2.14

•• = Only 2 animals in this group
* = Calculated from data less than 30 dpm above background
** = Calculated from data less than 10 dpm above background

TABLE 6

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Cumulative Excretion of Total Radioactivity from 10 Rats for the Period 0-96 h Following a Single Intravenous Dose of ^{14}C-HMX at 2 mg.kg^{-1}
(Results Expressed as % Dose)

Time (h)	46♂	47♂	50♂	52♂	54♂	63♀	64♀	68♀	69♀	71♀	Mean
Urine											
0-6	20.72	34.17	25.52	24.99	28.59	26.79	25.61	14.61	14.43	14.88	23.03
0-24	56.23	52.77	57.51	55.35	59.04	60.04	63.12	48.78	42.55	49.92	54.53
0-48	58.73	55.24	60.04	58.65	62.86	64.46	68.61	56.33	47.07	56.95	58.89
0-72	59.77	56.10	60.98	59.67	63.73	65.26	70.27	58.67	48.61	61.83	60.49
0-96	60.15	56.39	61.39	60.09	64.04	65.54	70.69	59.25	48.95	62.48	60.89
Faeces											
0-24	0.85	1.30	1.92	1.28	1.19	2.16	1.49	2.90	0.73	1.65	1.55
0-48	1.21	1.40	2.43	1.47	1.41	2.89	2.03	4.23	4.20	2.28	2.36
0-72	1.42	1.64	2.51	1.61	1.50	3.14	2.16	5.20	4.80	2.47	2.65
0-96	1.60	1.67	2.58	1.64	1.58	3.33	2.24	5.64	4.91	2.59	2.76
Cage Wash	0.81	0.59	2.25	1.21	1.95	1.81	1.29	3.64	1.30	2.81	1.77
Carcass	5.85	6.47	6.09	6.32	6.05	4.29	4.84	3.72	3.42	4.49	4.70
GI Tract	0.37	0.49	0.42	0.40	0.36	0.33	0.33	0.34	0.23	0.34	0.36
$^{14}CO_2$	-	7.05	-	-	-	4.77	-	-	-	-	-
Total Excreted											
0-6	20.72	34.17	25.52	24.99	28.59	26.76	25.61	14.61	14.43	14.88	23.03
0-24	57.08	54.07	59.43	56.63	60.23	62.20	64.59	51.68	43.28	51.57	55.78
0-48	59.94	56.64	62.47	60.12	64.27	67.35	70.64	60.56	51.28	59.23	61.26
0-72	61.19	57.74	63.49	61.28	65.23	68.40	72.43	63.87	53.41	64.30	63.14
0-96	61.75	58.06	63.97	61.73	65.62	68.87	72.93	64.71	53.86	65.07	63.65
Total	68.78	78.66+	72.73	69.66	73.93	80.07+	79.39	72.41	58.81	72.71	

+ = Includes $^{14}CO_2$

* = Includes carcass and cage wash

TABLE 7

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Cumulative Excretion of Total Radioactivity Following Intravenous Administration of ^{14}C-HMX to 5 Male and 5 Female Rats (2 mg.kg^{-1})
(Results Expressed as % Dose Recovered, Mean ± S.D. of 5 Rats Unless Otherwise Stated)

Time (h)	Male	Female
Urine		
0- 6	26.8 ± 5.0	19.3 ± 6.3
0-24	56.12 ± 3.3	52.9 ± 8.50
0-48	59.10 ± 3.5	58.7 ± 8.3
0-72	60.05 ± 3.5	60.9 ± 8.1
0-96	60.4 ± 3.5	61.4 ± 8.1
Faeces		
0-24	1.3 ± 0.4	1.8 ± 0.8
0-48	1.6 ± 0.5	3.1 ± 1.0
0-72	1.7 ± 0.4	3.6 ± 1.4
0-96	1.8 ± 0.4	3.7 ± 1.4
Cage Wash		
0-96	1.4 ± 0.7	2.2 ± 1.0
Gastro Intestinal Tract 96 h °	0.4 ± 0.1	0.3 ± 0.1
Carcass 96 h	6.2 ± 0.2	4.2 ± 0.6
Carcass Minus Organs 96 h †	4.7	3.1
Total Excreted		
0- 6	26.8 ± 5.0	19.3 ± 6.3
0-24	57.5 ± 3.5	54.7 ± 8.7
0-48	60.7 ± 3.8	61.8 ± 7.6
0-72	61.8 ± 3.7	64.5 ± 7.1
0-96	62.3 ± 3.7	65.1 ± 7.1
$^{14}CO_2$,		
0-24	5.4	4.2
0-48	7.1	4.8
Total (approx)	77.4	76.7

° = 3 rats † = mean 2 rats

TABLE 8

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:

Plasma Levels of Total Radioactivity Following Intravenous Administration of ^{14}C-HMX to 5 Male Rats

(Results Expressed as ng Equiv.ml^{-1} Normalised to the Target Dose of 2.0 mg.kg^{-1} Dose)

Time	Animal Number					Mean ± S.D.
	38♂	39♂	40♂	41♂	43♂	
2 min	774	670	657	746	810	731 ± 66
7 min	778	782	775	710	800	769 ± 34
15 min	898	996	878	696	911	876 ± 110
30 min	994	893	831	881	938	907 ± 61
1.0 h	1084	1219	942	1107	1071	1085 ± 99
2.0 h	1205	1414	998	1153	1230	1200 ± 150
4.0 h	1023	1182	930	1068	1113	1063 ± 95
6.0 h	946	1224	864	1182	1143	1072 ± 158
24 h	216	233	189	213	223	215 ± 16
48 h	87	102	73	90	81	87 ± 11
72 h	48	55	40	56	53	50 ± 7

TABLE 9

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Plasma Levels of Total Radioactivity Following Intravenous Administration of ^{14}C-HMX to 5 Female Rats
(Results Expressed as ng Equiv.ml^{-1} Normalised to the Target Dose of 2.0 mg.kg^{-1} Dose)

Time	Animal Number					Mean ± S.D.
	59♀	60♀	62♀	65♀	72♀	
2 min	686	152	824	413	76	430 ± 325
7 min	667	196	740	•	85	
15 min	744	229	913	595	132	523 ± 334
30 min	762	253	837	501	156	502 ± 300
1.0 h	810	268	955	581	209	564 ± 327
2.0 h	745	336	905	559	266	562 ± 269
4.0 h	804	437	891	489	332	591 ± 243
6.0 h	704	555	807	445	392	581 ± 174
24 h	232	293	248	162	303	247 ± 56
48 h	90	107	95	54	122	94 ± 25
72 h	48	54	57	33	60	51 ± 11

• = Sample error, insufficient sample remaining for reanalysis

TABLE 10

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue Distribution of Total Radioactivity 2 min After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})
(Results Expressed as % Dose g^{-1} and ng.Equiv g^{-1} Tissue)

Tissue	Animal Number							
	84♂		78♂		89♀*		90♀	
	% dose g^{-1}	ng.equiv g^{-1}	% dose g^{-1}	ng.equiv g^{-1}	% dose g^{-1}	ng.equiv g^{-1}	% dose g^{-1}	ng equiv. g^{-1}
Whole Blood	0.726	1840	0.881	2181	2.716	1536	1.019	2418
Plasma	0.481	1218	0.680	1682	1.335	755	0.771	1829
Heart	2.070	5244	1.967	4867	2.592	1466	2.262	5369
Lung	2.050	5195	10.063	24894	3.261	1844	6.769	16068
Liver	1.306	3310	1.856	4590	1.427	807	2.046	4857
Kidney	0.911	2309	1.876	4640	2.130	1205	2.080	4938
Spleen	0.469	1189	0.722	1787	1.080	611	1.111	2638
Thymus	0.552	1398	0.750	1854	0.582	329	0.830	1970
Brain	0.084	213	0.090	223	0.104	59	0.095	226
Testes and Seminal Vesicles	0.092	232	0.084	207	-	-	-	-
Ovaries and Uterus	-	-	-	-	0.531	300	0.854	2026
Skeletal Muscle	0.440	1116	0.543	1344	0.425	240	0.583	1384
Fat	0.112	284	0.199	492	0.209	118	0.208	664
Bone	0.440	1115	0.502	1243	0.547	309	0.583	1384
GI Tract	0.411	1042	0.516	1276	0.480	271	0.652	1547
Remaining Carcass	0.593	1503	0.571	1413	0.500	283	0.525	1245

* = Note that the dose received in this animal was significantly lower than target

TABLE 11

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue Distribution of Total Radioactivity 24 h After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})
(Results Expressed as % Dose g^{-1} and ng Equiv.g^{-1} Tissue)

Tissue	Animal Number							
	83♂		94♂		87♀		86♀	
	% dose g^{-1}	ng equiv. g^{-1}	% dose g^{-1}	ng equiv. g^{-1}	% dose g^{-1}	ng equiv. g^{-1}	% dose g^{-1}	ng equiv. g^{-1}
Whole Blood	0.075	211	0.102	221	0.084	235	0.078	214
Plasma	0.060	170	0.083	180	0.071	199	0.064	176
Heart	0.119	335	0.097	273	0.113	316	0.110	303
Lung	0.081	226	0.137	385	0.135	380	0.138	380
Liver	0.343	962	0.287	804	0.283	794	0.269	745
Kidney	0.309	886	0.251	705	0.228	640	0.257	707
Spleen	0.166	466	0.137	386	0.153	430	0.140	385
Thymus	0.231	649	0.198	557	0.238	669	0.215	592
Brain	0.017	47	0.040	86	0.040	112	0.037	103
Testes and Seminal Vesicles	0.038	106	0.136	295	-	-	-	-
Ovaries and Uterus	-	-	-	-	0.107	299	0.113	310
Skeletal Muscle	0.058	162	0.092	199	0.089	249	0.091	251
Fat	0.076	213	0.091	198	0.103	288	0.116	319
Bone	0.124	348	0.113	245	0.087	244	0.156	429
GI Tract	0.105	296	0.109	236	0.124	348	0.117	323
Remaining Carcass	0.065	183	0.190	411	0.093	262	0.091	251

TABLE 12

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue Distribution of Total Radioactivity 96 h After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})
(Results Expressed as % Dose g^{-1} and ng.Equiv g^{-1} Tissue)

Tissue	Animal Number							
	50♂		54♂		69♀		71♀	
	% dose g^{-1}	ng.equiv g^{-1}	% dose g^{-1}	ng equiv. g^{-1}	% dose g^{-1}	ng equiv. g^{-1}	% dose g^{-1}	ng equiv. g^{-1}
Whole Blood	0.034	89	0.029	76	0.016	43	0.022	58
Plasma	0.022	59	0.018	47	0.011	30	0.016	43
Heart	0.057	151	0.054	144	0.032	86	0.053	139
Lung	0.080	212	0.075	199	0.055	147	0.075	198
Liver	0.123	325	0.091	240	0.092	243	0.093	245
Kidney	0.175	461	0.156	413	0.081	216	0.139	365
Spleen	0.077	203	0.073	195	0.048	128	0.074	194
Thymus	0.133	350	0.103*	275*	0.077	203	0.135	356
Brain	0.017	44	0.014	38	0.008	23	0.014	37
Testes and Seminal Vesicles	0.036	94	0.031	83	-	-	-	-
Ovaries and Uterus	-	-	-	-	0.040	106	0.074	195
Skeletal Muscle	0.035	93	0.037	98	0.018	47	0.029	75
Fat	0.043	114	0.046	123	0.018	48	0.040	106
Bone	0.046	120	0.040	105	0.028	75	0.035	92
GI Tract	0.049	129	0.022	59	0.016	41	0.031	82
Remaining Carcass	0.046	121	0.045	120	0.024	64	0.037	96

* = Value outside Quality Control standard, insufficient sample for reanalysis

TABLE 13

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Retention of Total Radioactivity at Various Times After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1}).
(Results Expressed as % of Administered Dose)

Animal No./Sex	Time After Dosing	%* in Carcass	% in GI Tract	Total % Recovered	Mean % ± S.D.
84♂	2 min	85.99	6.68	92.67	90.3
78♂		88.24	7.16	95.50	(± 4.61)
89♀		79.36	6.23	85.56	
90♀		78.75	8.68	87.43	
83♂	24 h	9.94	1.62	11.56	17.1
94♂		25.84	1.85	27.69	(±7.2)
87♀		12.54	1.67	14.21	
86♀		12.96	1.80	14.76	
50♂	96 ht	6.09	0.42	6.51	5.4
54♂		6.05	0.36	6.41	(±1.47)
59♀		3.42	0.23	3.65	
71♀		4.49	0.34	4.83	

* = Includes residual carcass plus organs, tissues and blood removed for separate analysis

† = Animals used concurrently for excretion kinetics see Table 6

TABLE 14

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue:Plasma Ratios of Total Radioactivity 2 min After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})

Tissue	Animal Number and Sex				Mean ± S.D.
	84♂	78♂	89♀	90♀	
Whole Blood	1.51	1.30	2.03	1.32	1.5 ± 0.34
Heart	4.31	2.89	1.94	2.94	3.0 ± 0.98
Lung	4.26	14.80	2.44	8.79	7.6 ± 5.51
Liver	2.72	2.73	1.07	2.66	2.3 ± 0.82
Kidney	1.90	2.76	1.60	2.70	2.2 ± 0.58
Spleen	0.98	1.06	0.81	1.44	1.1 ± 0.27
Thymus	1.15	1.10	0.44	1.08	0.9 ± 0.34
Brain	0.17	0.13	0.08	0.12	0.1 ± 0.04
Testes and Seminal Vesicles	0.19	0.12	-	-	0.2
Ovaries and Uterus	-	-	0.40	1.11	0.8
Skeletal Muscle	0.92	0.80	0.32	0.76	0.7 ± 0.26
Fat	0.23	0.29	0.16	0.27	0.2 ± 0.06
Bone	0.92	0.74	0.41	0.76	0.7 ± 0.21

TABLE 15

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue:Plasma Ratios of Total Radioactivity 24 h After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})

Tissue	Animal Number and Sex				Mean ± S.D.
	83♂	94♂	87♀	86♀	
Whole Blood	1.24	1.23	1.18	1.22	1.2 ± 0.03
Heart	1.97	1.52	1.59	1.72	1.7 ± 0.20
Lung	1.33	1.64	1.91	2.16	1.8 ± 0.36
Liver	5.66	4.47	3.99	4.23	4.6 ± 0.74
Kidney	5.21	3.92	3.22	4.02	4.1 ± 0.83
Spleen	2.74	2.14	2.16	2.19	2.3 ± 0.29
Thymus	3.82	3.09	3.36	3.36	3.4 ± 0.30
Brain	0.28	0.48	0.56	0.59	0.5 ± 0.14
Testes and Seminal Vesicles	0.62	1.64	-	-	1.1
Ovaries and Uterus	-	-	1.50	1.76	1.6
Skeletal Muscle	0.93	1.11	1.25	1.43	1.2 ± 0.21
Fat	1.25	1.10	1.45	1.81	1.4 ± 0.31
Bone	2.05	1.36	1.23	2.44	1.8 ± 0.57

TABLE 16

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Tissue:Plasma Ratios of Total Radioactivity 96 h After Intravenous Administration of ^{14}C-HMX to the Rat (Target Dose 2.0 mg.kg^{-1})

Tissue	Animal Number and Sex				Mean ± S.D.
	50♂	54♂	67♀	71♀	
Whole Blood	1.51	1.62	1.43	1.35	1.5 ± 0.12
Heart	2.56	3.06	2.87	3.23	2.9 ± 0.29
Lung	3.59	4.23	4.90	4.60	4.3 ± 0.56
Liver	5.51	5.11	8.10	5.70	6.1 ± 1.35
Kidney	7.81	8.79	7.20	8.49	8.1 ± 0.71
Spleen	3.44	4.15	4.27	4.51	4.1 ± 0.46
Thymus	5.93	5.85	6.77	8.28	6.7 ± 1.13
Brain	0.75	0.81	0.77	0.86	0.8 ± 0.05
Testes and Seminal Vesicles	1.59	1.77	-	-	1.7
Ovaries and Uterus	-	-	3.53	4.53	4.0
Skeletal Muscle	1.58	2.09	1.57	1.72	1.7 ± 0.24
Fat	1.93	2.62	1.60	2.47	2.2 ± 0.47
Bone	2.03	2.23	2.50	2.14	2.2 ± 0.20

TABLE 17a

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Levels of ^{14}C-HMX Detected in Pooled Urine and Faeces Extracts Following Oral Administration to Rat and Mouse
Thin Layer Chromatography (petroleum ether:acetone:acetone 60:35:30 v/v)

% Total Radioactivity in Extract

TLC Zone	Urine			
	Rat (6-24 h)		Mouse (6-24 h)	
	♂	♀	♂	♀
Polar (origin)	7.9	8.0	5.2	5.2
I	0.6	0.5	2.1	1.0
II	88.9	58.2	7.5	7.4
HMX	2.7	33.3	85.1	86.4
	100.1	100.0	99.9	100.0

TLC Zone	Faeces			
	Rat (0-24 h)		Mouse (0-24 h)	
	♂	♀	♂	♀
Polar (origin)	0.2	0.1	0.4	0.5
I	0.2	0.2	0.2	0.0
II	1.1	2.3	1.7	0.1
HMX	98.5	97.4	97.8	99.4
	100.0	100.0	100.1	100.0

I = Region above HMX (less polar)
II = Region below HMX but above origin (more polar)

TABLE 17b

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Levels of ^{14}C-HMX Detected in Pooled Urine and Faeces Extracts Following Oral Administration to Rat and Mouse
Thin Layer Chromatography (petroleum ether:acetone:acetonitrile 60:35:30 v/v)

(Results† are Expressed as % Dose Eliminated cf Table 2)

TLC Zone	Urine			
	Rat (6-24 h)		Mouse (6-24 h)	
	♂	♀	♂	♀
Polar (origin)	0.1	0.1	0.0	0.1
I	0.0	0.0	0.0	0.0
II	1.4	0.8	0.1	0.1
HMX	0.0	0.5	0.6	1.5
% Dose	1.5	1.4	0.7	1.7

TLC Zone	Faeces			
	Rat (0-24 h)		Mouse (0-24 h)	
	♂	♀	♂	♀
Polar (origin)	0.1	0.1	0.2	0.3
I	0.1	0.1	0.1	0.0
II	0.8	1.3	0.9	0.1
HMX	70.0	58.5	50.8	66.9
% Dose	71.0	60.0	52.0	67.3

I = Region above HMX (less polar)
II = Region below HMX but above origin (more polar)
† = Assesses quantitative extraction

TABLE 18a

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
The Nature of Radioactivity in Urine Separately Pooled for Male and Female Rats at Various Times after Intravenous Administration of ^{14}C-HMX
(Target Dose 2 mg.kg^{-1})
(Results Expressed as % of the Total Radioactivity Recovered in the Acetonitrile Extract and Residue)

Component	TLC approx Rf value	0-6 h Urine		6-24 h Urine		24-48 h Urine	
		♂	♀	♂	♀	♂	♀
Unchanged HMX	0.51	52.4	64.2	33.2	66.9	16.9	50.2
Metabolite 1	0.08	0.8	1.0	0.1	0.0	0.0	0.0
Metabolite 2	0.03	1.5	1.9	1.8	1.8	2.1	0.9
Origin	0.0-0.01	40.7	30.5	50.6	25.8	75.6	36.0
Remainder		0.9	1.0	1.0	2.3	0.4	6.1
% Extracted		96.4	98.3	86.6	96.9	95.0	93.1
% Residue		3.6	1.7	13.4	3.1	5.0	6.9
Total		100.0	100.0	100.0	100.0	100.0	100.0

TLC performed on silica gel plates (250 um thick) and developed in dichloromethane:acetonitrile 80:30. Unlabelled reference standards SEX, PDX and Tetryl had Rf values of 0.22, 0.61 and 0.76 respectively.

TABLE 18b

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
The Nature of Radioactivity in the Urine of Rats
Following Intravenous Administration of ^{14}C-HMX (2 $mg.kg^{-1}$)
(Results are Expressed as % Dose Eliminated cf Table 6)

	HMX (Rf 0.51)	Met 1 (Rf 0.08)	Met 2 (Rf 0.03)	Origin (0.0-0.01)	Others	Unextracted Material	Total
Urine from Male Rats							
0-6 h	14.0	0.2	0.4	10.9	0.2	1.0	26.8
6-24 h	10.0	0.0	0.5	15.2	0.3	4.0	30.1
24-48 h	0.5	0.0	0.1	2.2	0.0	0.1	2.9
Total	24.5	0.2	1.0	28.3	0.5	5.1	59.8
Urine from Female Rats							
0-6 h	12.4	0.2	0.3	5.9	0.2	0.3	19.3
6-24 h	22.5	0.0	0.6	8.7	0.8	1.0	33.6
24-48 h	2.9	0.0	0.1	2.1	0.4	0.4	5.8
Total	37.8	0.2	1.0	16.7	1.4	1.7	58.7

Thin layer chromatography: dichloromethane:acetonitrile: 8:3 (v/v)

Reference standards:		
	HMX	Rf 0.51
	SEX	Rf 0.22
	RDX	Rf 0.61
	Tetryl	Rf 0.76

TABLE 19

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Plasma Levels of Unchanged ^{14}C-HMX at 2 min and 24 h Following Intravenous Administration of ^{14}C-HMX to Rats (Target Dose 2 mg.kg^{-1})
Samples Processed by HPLC (see text for details)

Animal No.	Time of Sample	Proportion Extracted	Proportion HMX	Total Radioactivity (ng equiv.ml^{-1})	HMX (ng.g^{-1})
78♂	2 min	90	97	1682	1468
84♂		100*	98	1218	1194
89♀		100*	97	755	732
90♀		100*	96	1829	1756
83♂	24 h	14.9	80	170	20
94♂		24.5	41	181	18
86♀		30.7	47	176	25
87♀		30.7	43	199	26

* = Calculated exceeded 100%, 100% extraction figure used for calculation

TABLE 20a

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:

HPLC Analysis of Urine (and Faeces 0-24 h) ^{14}C from Rats up to 96 h after a Single Intravenous Dose of ^{14}C-HMX (2 mg.kg^{-1})

(Results Expressed as % of the Total Radioactivity Recovered in the Acetonitrile Extract and Residue)

Component	HPLC Rt min	Urine										Faeces	
		0-6 h		6-24 h		24-48 h		48-72 h		72-96 h		0-24 h	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Highly polar non-retained	4	40.4	27.9	50.6	27.0	74.7	39.5	81.9	45.9	74.7	46.6	14.6	14.2
Metabolite A	10	0.8	0.7	0.5	0.5	0.0	0.2	0.3	0.3	1.3	0.1	0.3	0.5
Metabolite B	15	0.4	0.2	0.5	0.6	0.2	0.9	0.4	0.4	0.2	1.0	4.0	2.6
Metabolite C	18	1.2	1.1	1.0	1.5	0.8	2.0	0.7	1.7	0.2	1.5	8.7	10.2
HMX	22	52.2	67.6	33.2	66.8	19.0	49.6	11.4	45.6	15.4	41.8	23.8	38.1
Remainder		1.4	0.8	0.8	0.3	0.3	0.8	0.5	0.3	1.1	1.0	0.9	0.6
% Extracted in acetonitrile		96.4	98.3	86.6	96.9	95.0	93.1	95.2	94.2	93.1	91.4	52.3	66.2
% Residue		3.6	1.7	13.4	3.1	5.0	6.9	4.8	5.8	6.9	8.6	47.7	33.8
Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Retention time of standards (min): HMX 22 min
SEX 10 min
RDX 19 min
Tetryl 71 min

TABLE 20b

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:

HPLC Analysis of Urine (and Faeces 0-24 h) ^{14}C from Rats up to 96 h after a Single Intravenous Dose of ^{14}C-HMX (2 mg.kg^{-1})

(Results Expressed as % Dose Administered)

Component	Urine												Faeces		Total	
	0-6 h		6-24 h		24-48 h		48-72 h		72-96 h		0-96 h		0-24 h			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Highly polar	10.8	5.4	15.2	9.0	2.1	2.3	0.8	1.0	0.3	0.2	29.2	17.9	0.2	0.2	29.4	18.1
Metabolite A	0.2	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.0	0.0	0.4	0.3
Metabolite B	0.1	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.0	0.4	0.2
Metabolite C	0.3	0.2	0.3	0.5	0.0	0.1	0.0	0.0	0.0	0.0	0.6	0.8	0.1	0.2	0.7	1.0
HMX	14.0	13.0	10.0	22.4	0.6	2.9	0.1	1.0	0.1	0.2	24.8	39.8	0.3	0.7	25.1	40.5
Remainder	0.4	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.3	0.0	0.0	0.6	0.3
Proportion unextracted	1.0	0.3	4.0	1.0	0.1	0.4	0.0	0.1	0.0	0.0	5.1	1.8	0.6	0.6	5.7	2.4
Total	26.8	19.3	30.1	33.6	2.9	5.8	1.0	2.2	0.4	0.5	61.2	61.4	1.3	1.8	62.5	63.2

TABLE 21

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Levels of HMX and Polar Metabolite Detected in Bodies of Male and Female Rats Sacrificed at 2 min, 24 and 96 h Following Intravenous Administration of ^{14}C-HMX (target dose 2 $mg.kg^{-1}$)

Sample	% Extraction ^{14}C from Tissue	% Recovery of ^{14}C from Column	% of Eluate as HMX	% of Eluate as Polar Metabolite	% of Original Tissue Radioactivity	
					HMX	Polar Metabolite
2 min						
Liver ♂	79.1	72.5	62.0	28.8	35.6	16.5
♀	75.4	72.3	45.7	39.1	24.9	21.3
Lung ♂	104.4*	99.1	97.5	0.3	96.6	0.3
♀	95.3	95.9	97.2	0.6	88.8	0.5
Kidney ♂	95.0	85.0	82.1	1.1	66.3	0.9
♀	96.1	100	88.8	10.4	85.3	10.0
Brain ♂	93.6	90.6	93.8	1.2	79.5	1.0
♀	108.4*	77.5	65.3	4.9	50.6	3.8
Skeletal Muscle ♂	99.4	90.7	88.2	0.8	79.5	0.7
♀	98.9	89.3	88.3	1.7	80.1	1.5
24 h						
Liver ♂	42.3	37.4	†	88.7	†	14.0
♀	54.0	59.5	†	55.0	†	17.7
Heart ♂	56.6	91.1	52.9	20.7	27.3	10.7
♀	•					
Kidney ♂	60.7	59.2	33.1	34.6	11.9	12.4
♀	59.5	100	43.7	21.4	26.0	12.7
96 h						
Liver ♂	•					
♀	25.3	26.0	†	63.4	†	4.2

* = Taken as 100% † = Nil detected • = Insufficient sample for analysis

FIGURE 1

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: The Elimination Kinetics of Radioactivity Following Oral Administration of ^{14}C-HMX (500 mg.kg^{-1}) to Rats and Mice

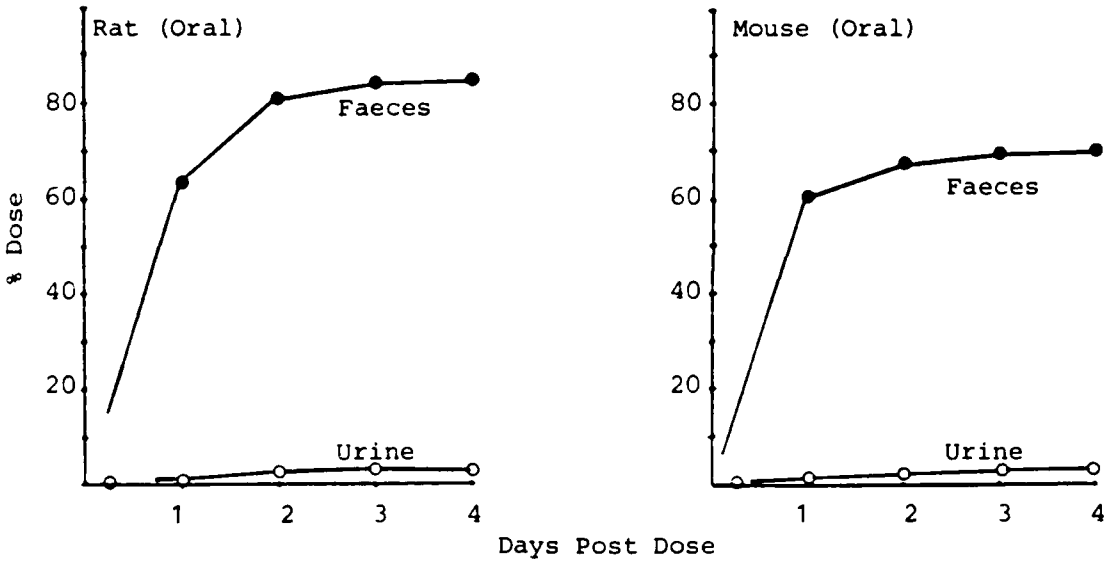


FIGURE 2

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Plasma Levels of Total Radioactivity Following a Single Oral Dose of ^{14}C-HMX (500 mg.kg^{-1}) to Rats and Mice
(Results Expressed as µg.Equiv.ml^{-1} Normalised to a Dose of 500 mg.kg^{-1})

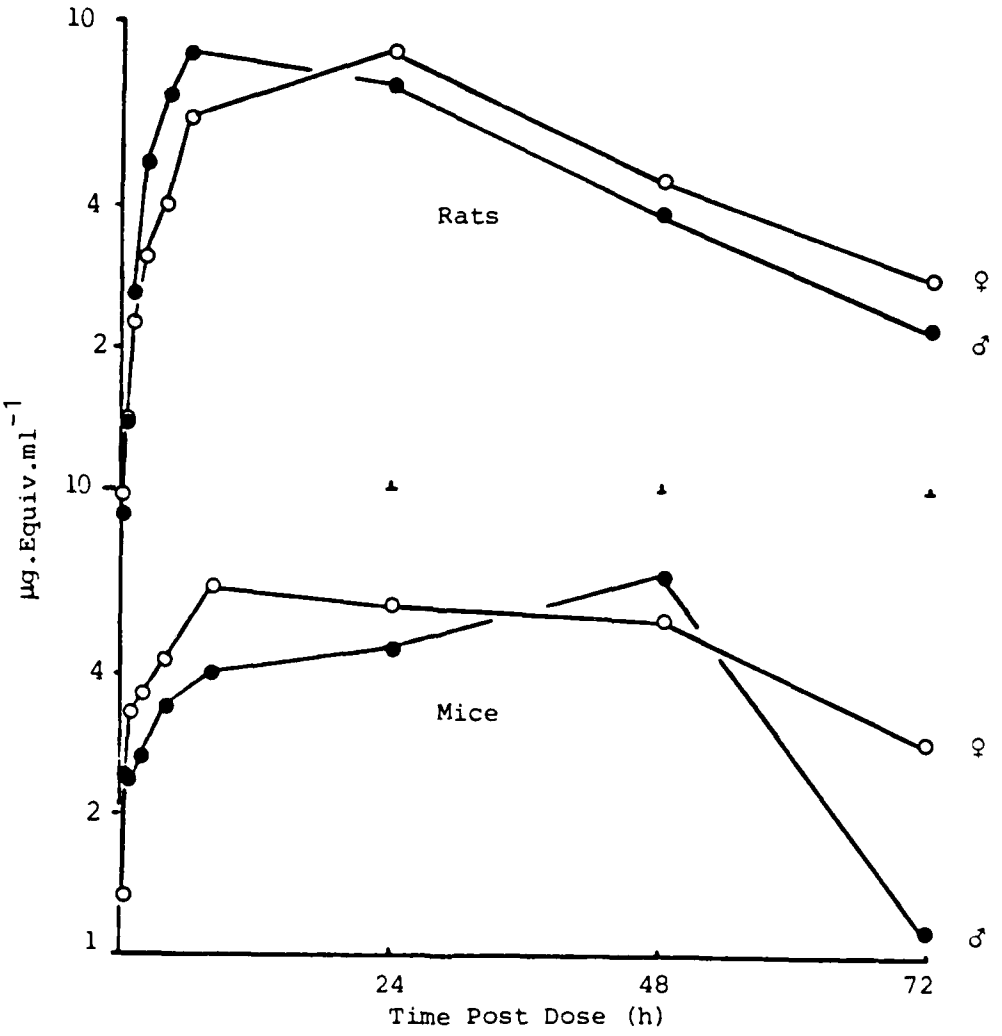


FIGURE 3

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: The Elimination Kinetics of Radioactivity Following Intravenous Administration of ^{14}C-HMX (2 mg.kg^{-1}) to Rats

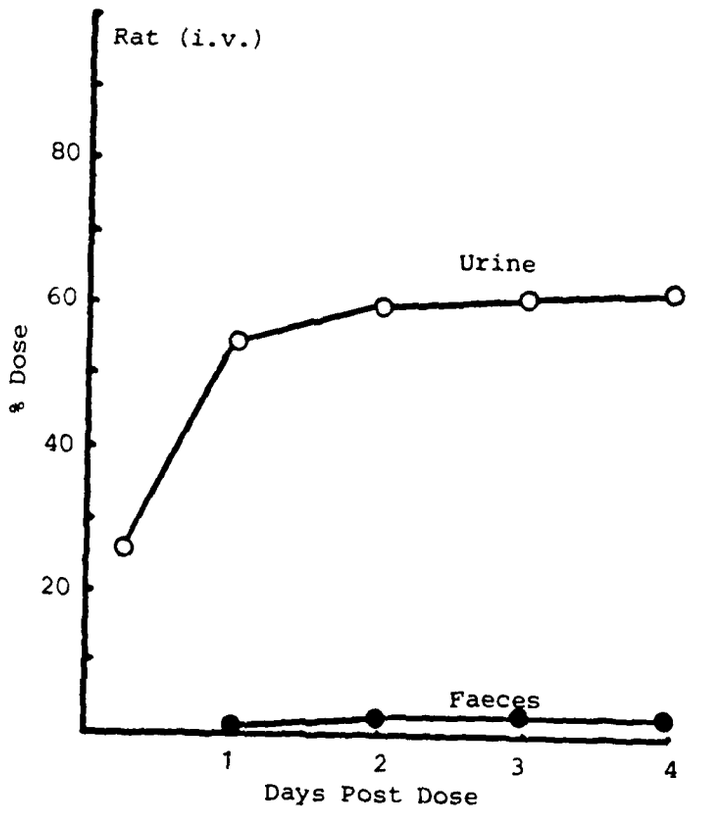


FIGURE 4

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration ot the Rat and Mouse and Intravenous Administration to the Rat: Plasma Levels of Radioactivity Following Intravenous Administration of ^{14}C-HMX (2 mg.kg^{-1}) to Male and Female Rats

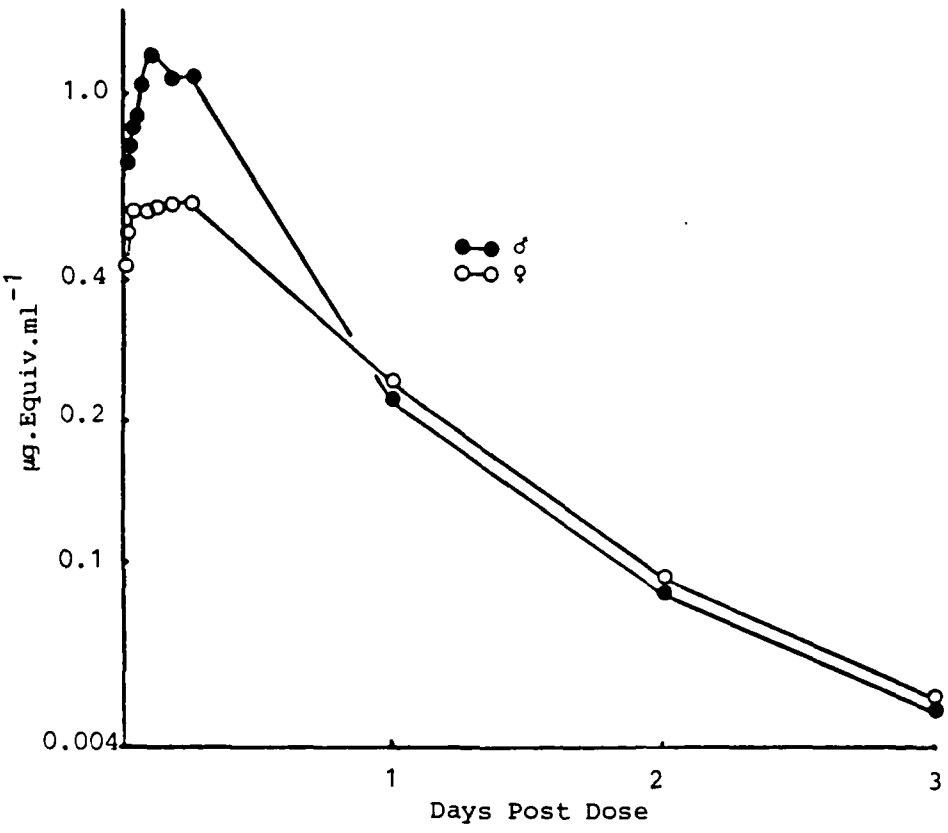
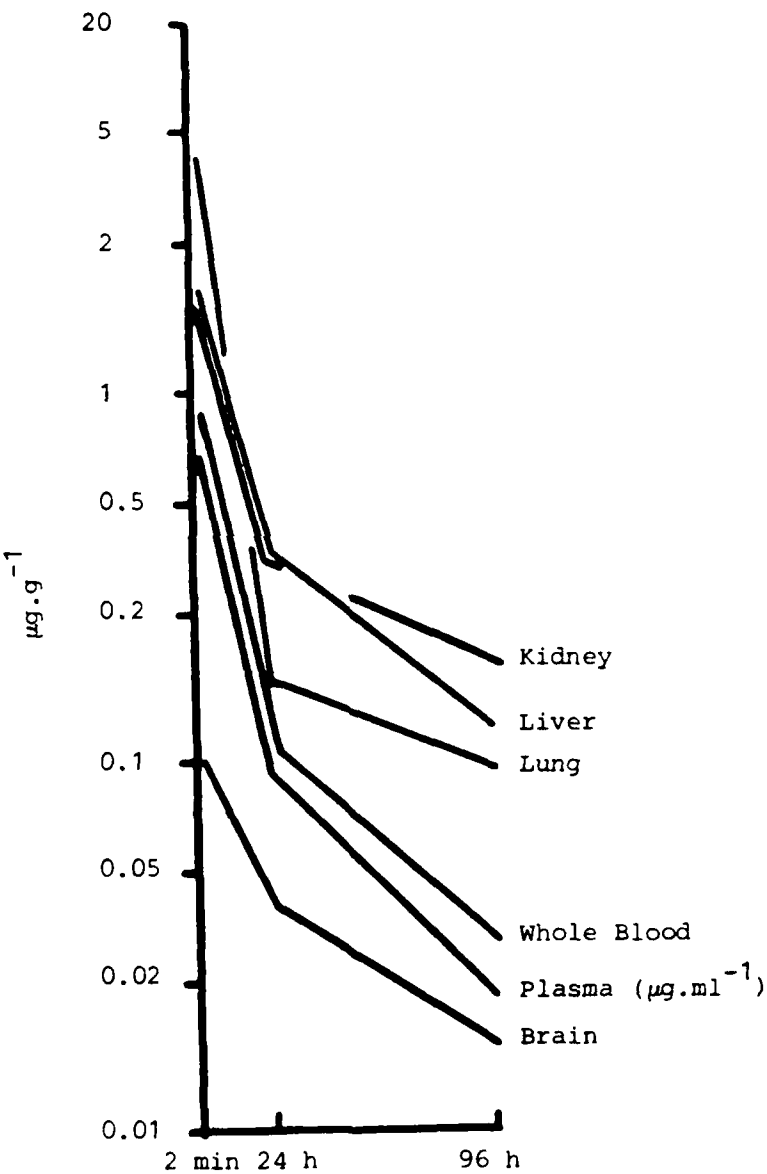


FIGURE 5

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Mean Concentration of Radioactivity in Selected Organs and Tissues at Times Following Intravenous Administration of ^{14}C-HMX (2 mg.kg^{-1}) to Male and Female Rats



Note: Mean results are taken from Tables 10,11 and 12. Animal No. 89 was excluded from the mean.

FIGURE 6

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Radioactivity in a Pooled Urine Sample from Female Rats Collected at 48-72 h Following a Single Intravenous Administration of ^{14}C-HMX (Target Dose 2 mg.kg^{-1}) Sample Co-injected with Non-Radioactive HMX as Marker

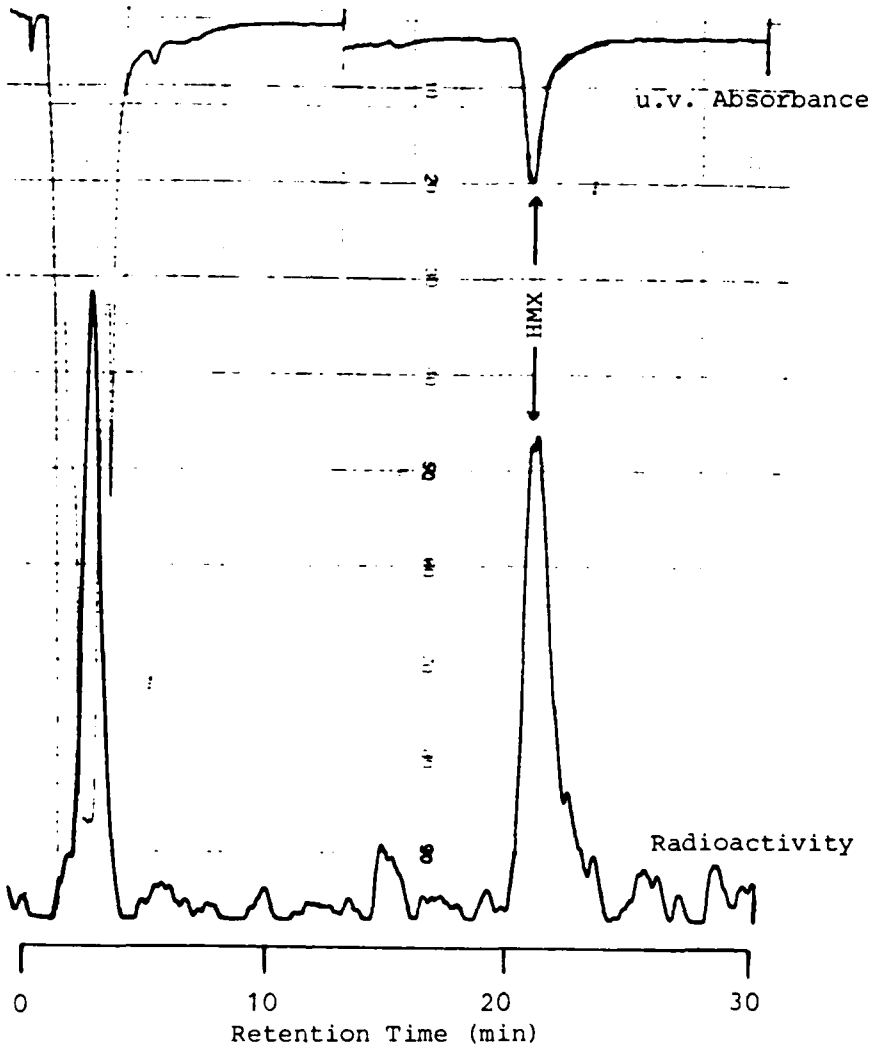


FIGURE 7

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Urine from Male Rats Collected 6-24 h After Intravenous Injection of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

Mobile Phase: A = 15% Acetonitrile, B = 40% Acetonitrile, C = 60% Acetonitrile } In water at pH 2.5 with SLS at 0.4%

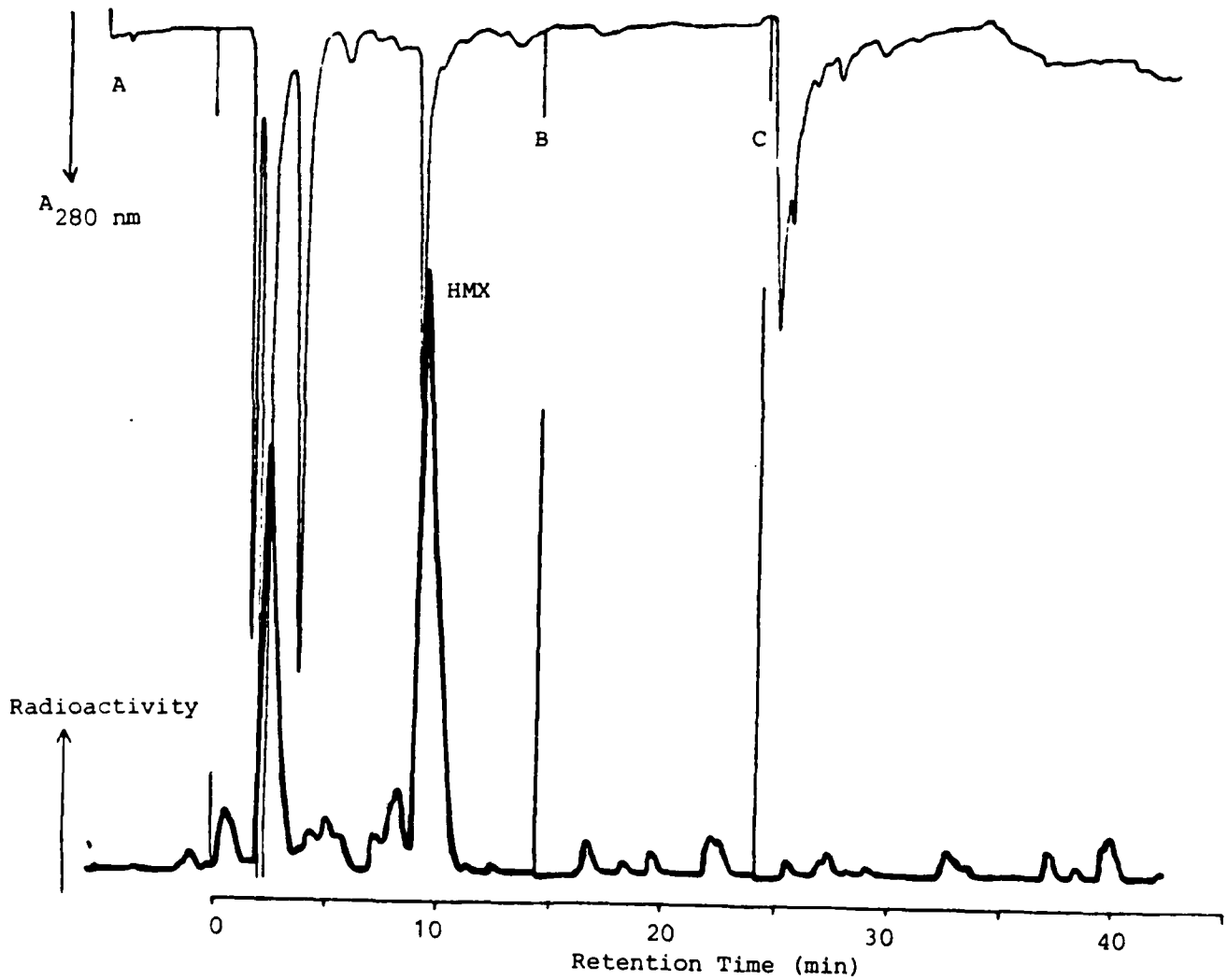


FIGURE 8

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Urine from Male Rats Collected 6-24 h After Intravenous Injection of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

Mobile Phase: A = 4% Acetonitrile } In water at pH 2.5
B = 60% Acetonitrile } with SLS at 0.4%

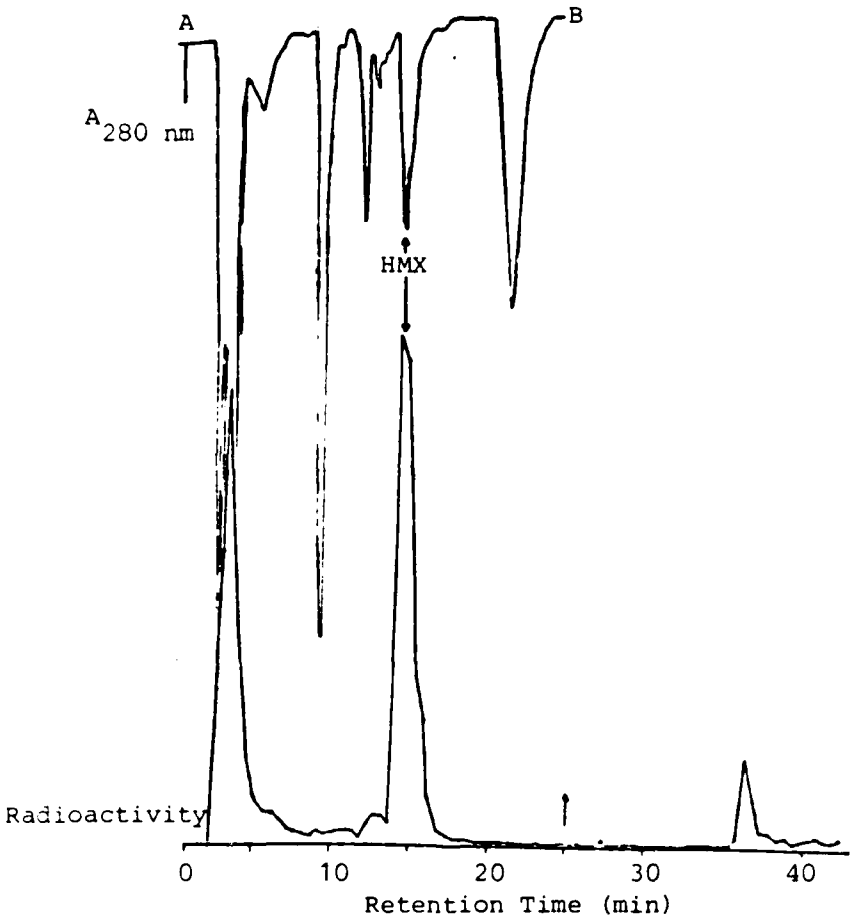


FIGURE 9

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Urine from Female Rats Collected 6-24 h After Intravenous Injection of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

Mobile Phase: A = 4% Acetonitrile } In water at pH 2.5
B = 60% Acetonitrile } with SLS at 0.4%

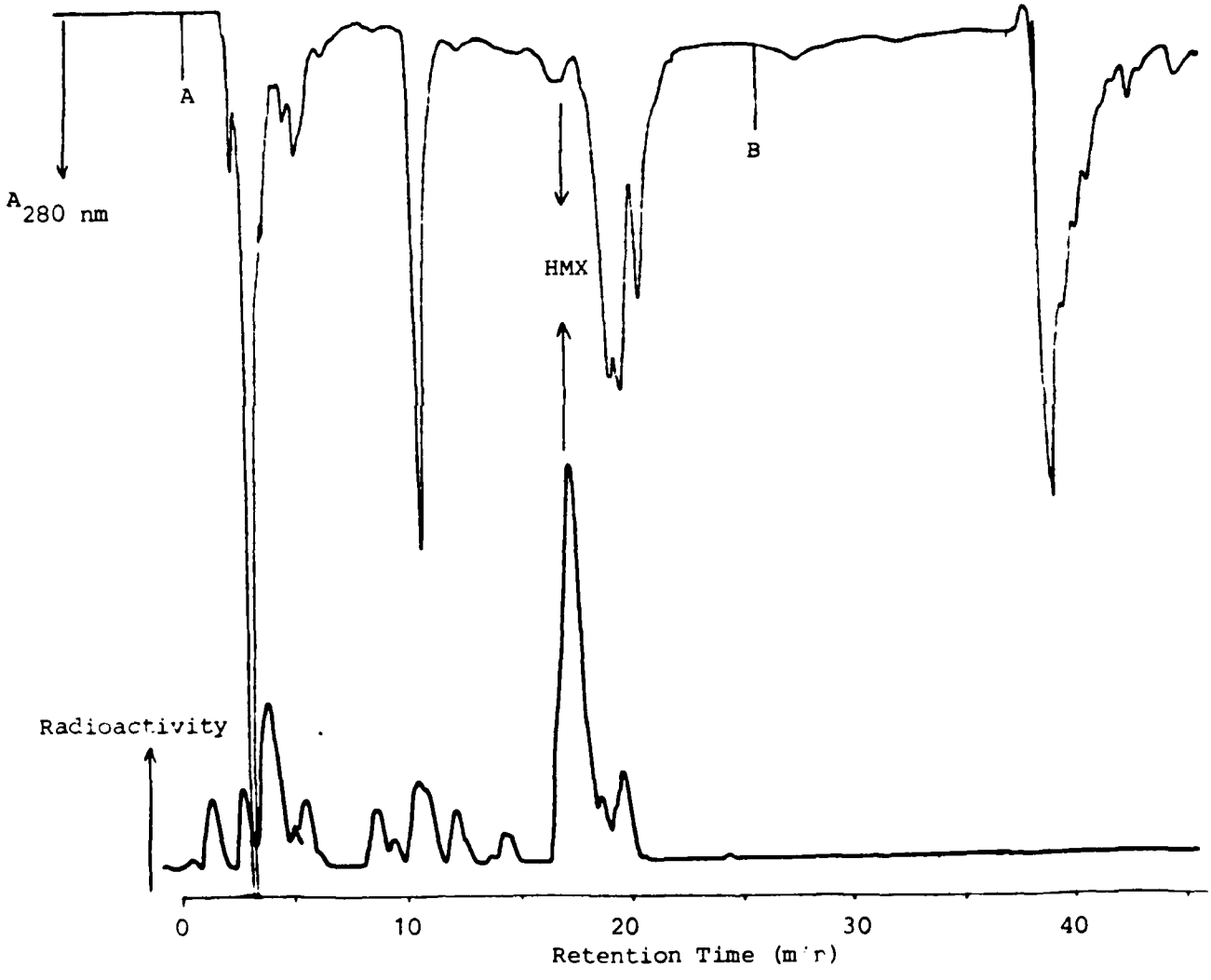


FIGURE 10

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Urine from Male Rats Collected 24-48 h After Intravenous Injection of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

Mobile Phase: A = Water pH 2.5
B = 15% Acetonitrile } In water at
C = 67% Acetonitrile } pH 2.5

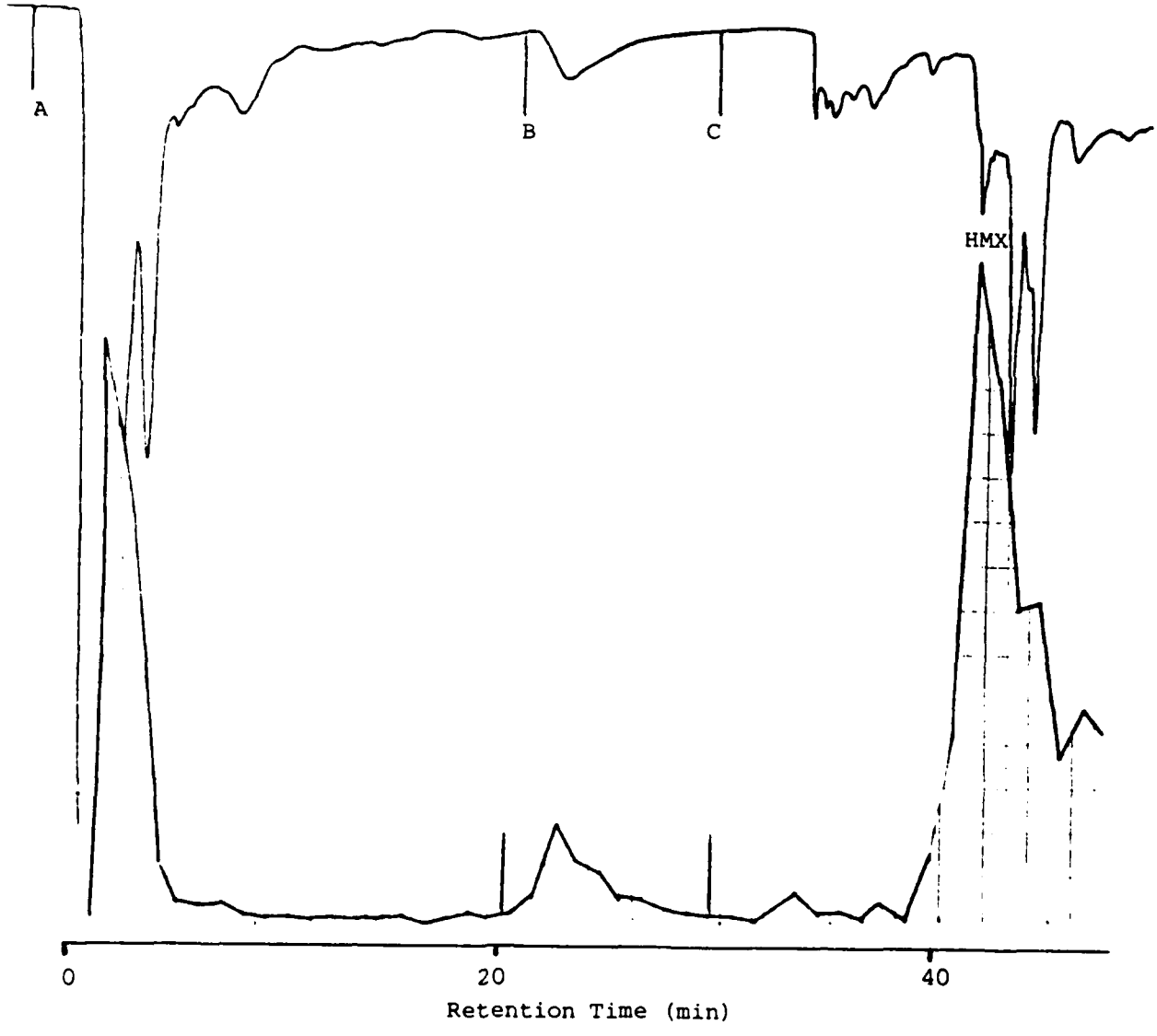


FIGURE 11

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Influence of Acid-hydrolysis on the Radio-HPLC Profile of Male Rat Urine Collected 6-24 h After a Single Intravenous Dose of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

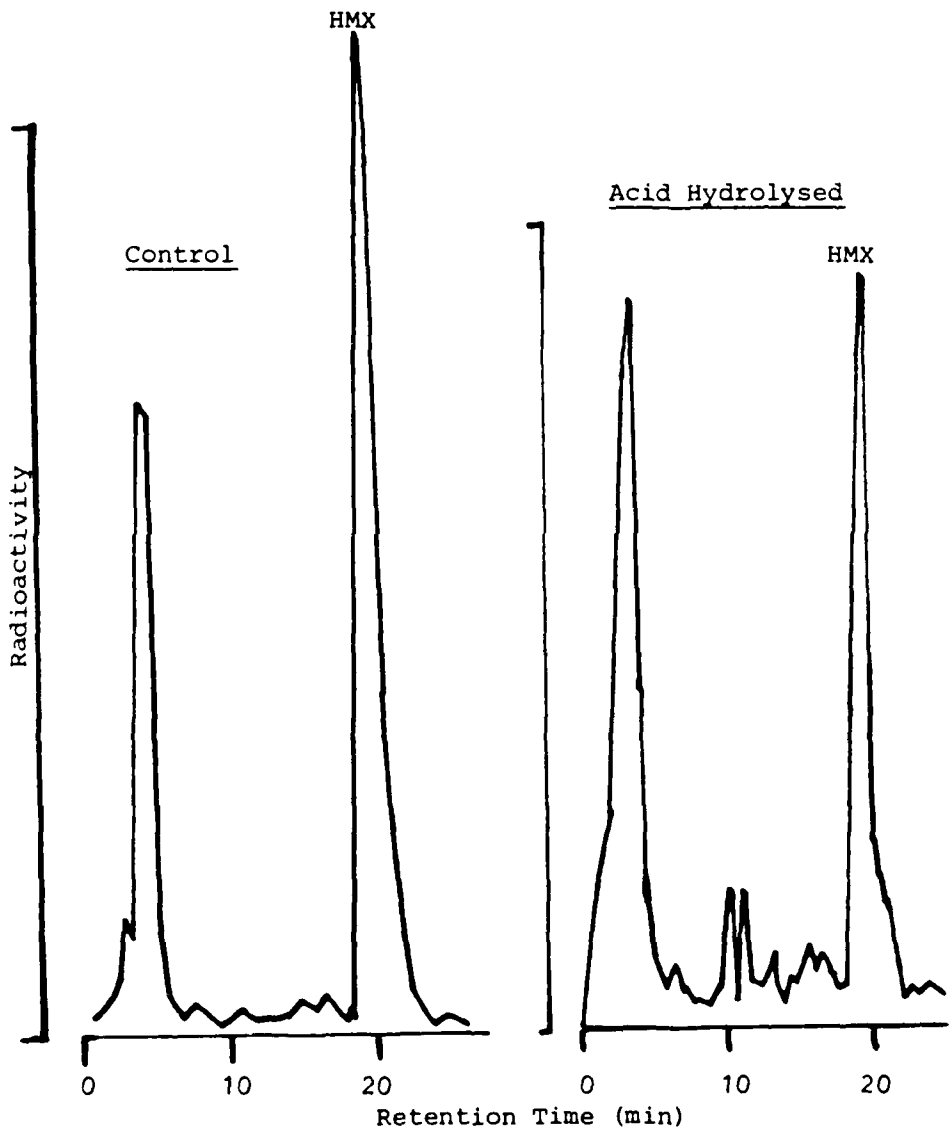


FIGURE 12

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Pooled Male Liver Extract Collected from Rats Sacrificed at 2 min Following Intravenous Administration of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})

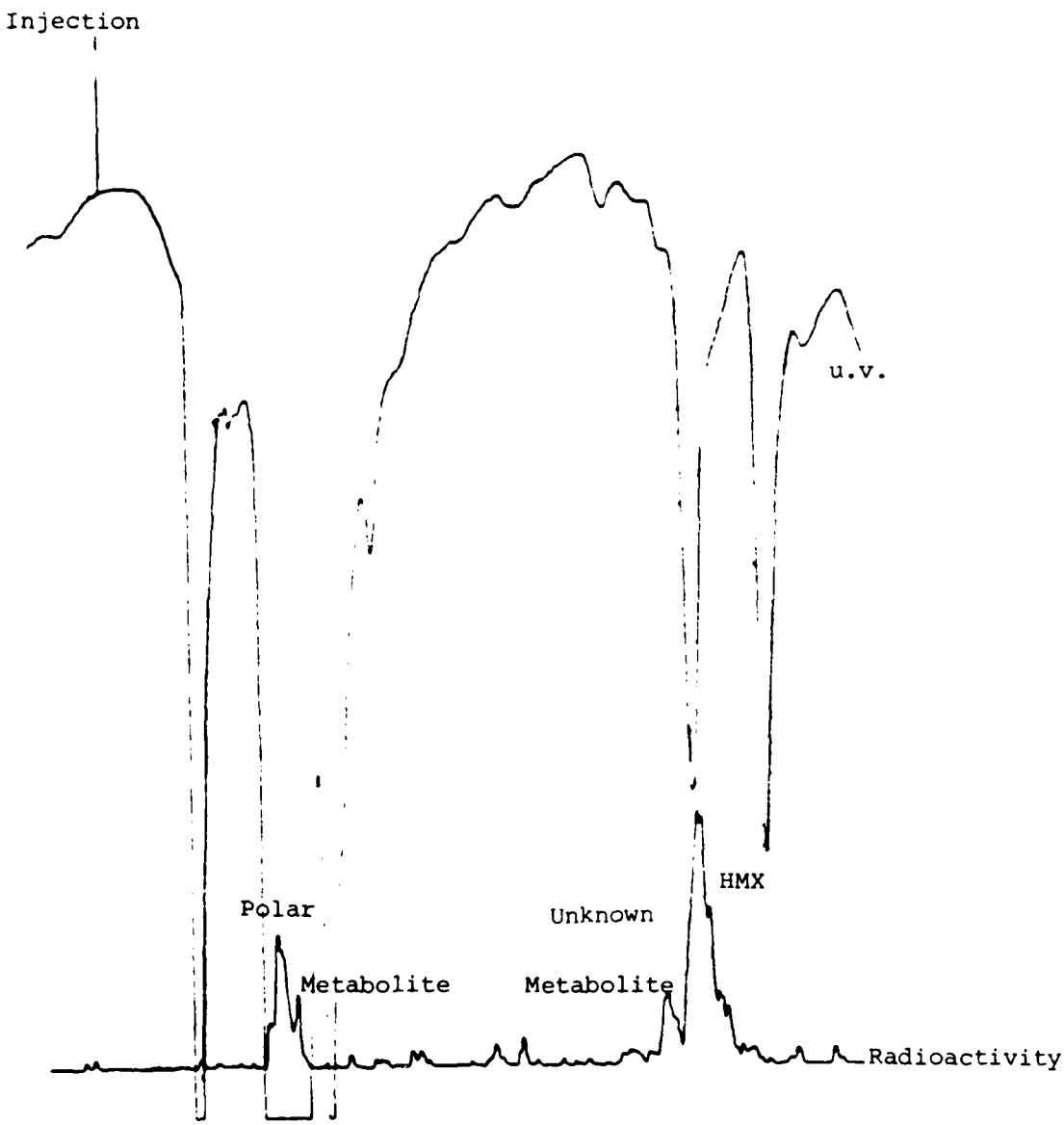
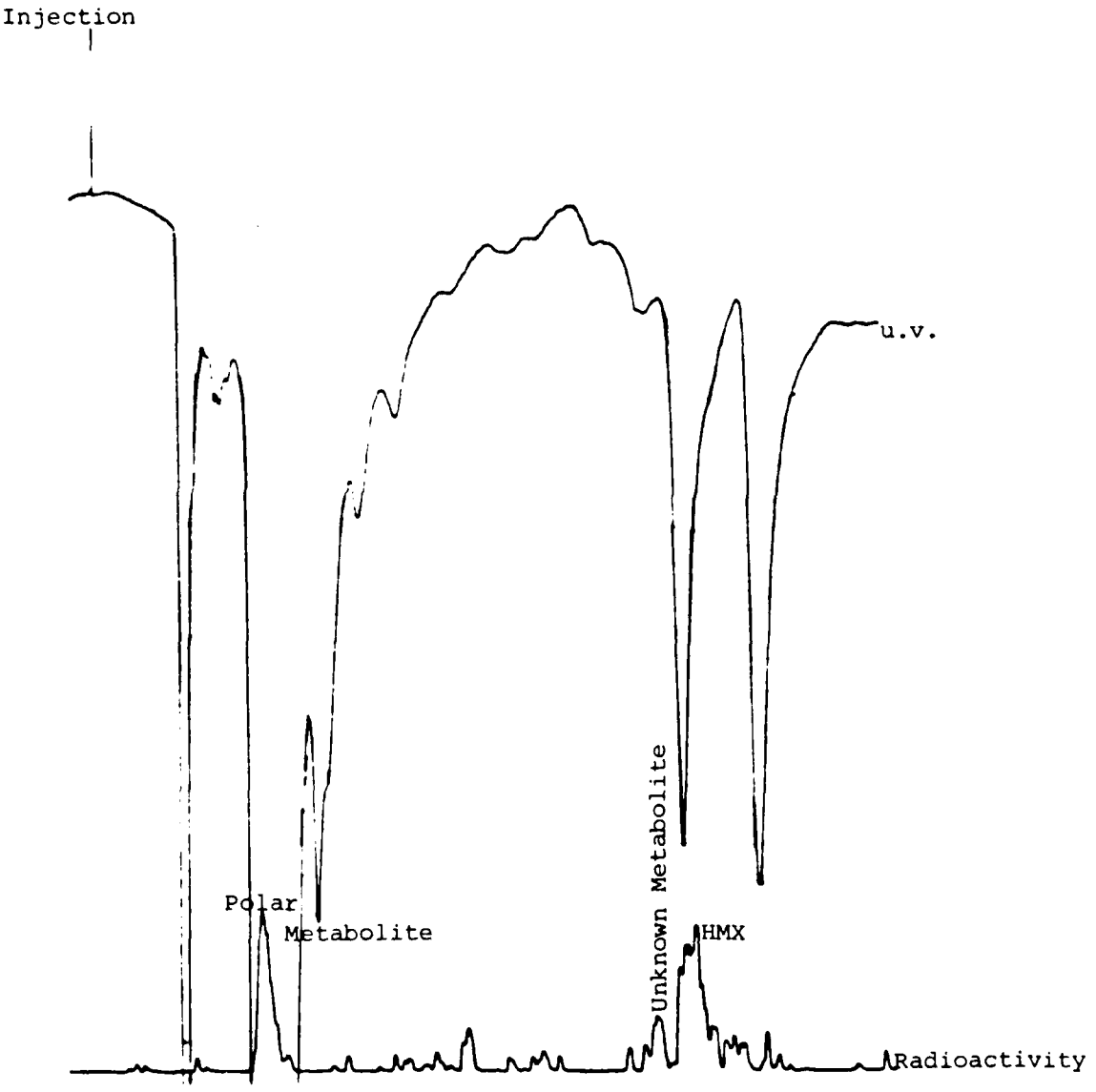


FIGURE 13

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: Radio-HPLC Profile of Pooled Female Liver Extract Collected from Rats Sacrificed at 2 min Following Intravenous Administration of ^{14}C-HMX (Target Dose 2 mg.kg^{-1})



APPENDIX 1

Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat: ^{14}C-HMX Data Sheet

NEN New England Nuclear

Radiochemical Specifications

CAUTION, NOT FOR USE IN HUMANS OR CLINICAL DIAGNOSIS: This product is intended for research or manufacturing use only. It is pharmaceutically unrefined and verification of its suitability for use in humans or as a clinical diagnostic reagent and the compliance with all Federal and State laws regulating such applications is the sole responsibility of the purchaser.

CUSTOM SYNTHESIS

Name of Compound : "HMX" OCTAHYDRO-1,3,5,7-TETRANITRO-1,3,5,7-TETRAZOCINE[^{14}C(U)]

Chemical Formula : $C_4 H_8 N_8 O_8$

Lot No. : 1221-068 Assay No. 170535

Physical Form : CRYSTALLINE SOLID

Packaging Information : SCREW CAP BOTTLE

Radioactivity : 1.87 MILLICURIES

Weight : 93.3 MILLIGRAMS

Specific Activity : 5.93 MILLICURIES/MILLIMOLE

SPECIAL INFORMATION : DRY ICE SHIPPMENT.

Date : August 21, 1980

IMPORTANT NOTICE. The purity of the custom synthesis described herein has been demonstrated by the analytical methods listed. If the purity agreed upon by New England Nuclear Corporation and the purchaser per a formal quotation is not achievable, due to unforseen problems encountered in the synthesis and or purification, the purchaser will be so notified prior to shipment of the material. The stability of custom synthesized products is unknown and cannot be guaranteed beyond 15 days from receipt. The purchaser is urged to verify the purity of the material within this period.

RADIOCHROMATOGRAM

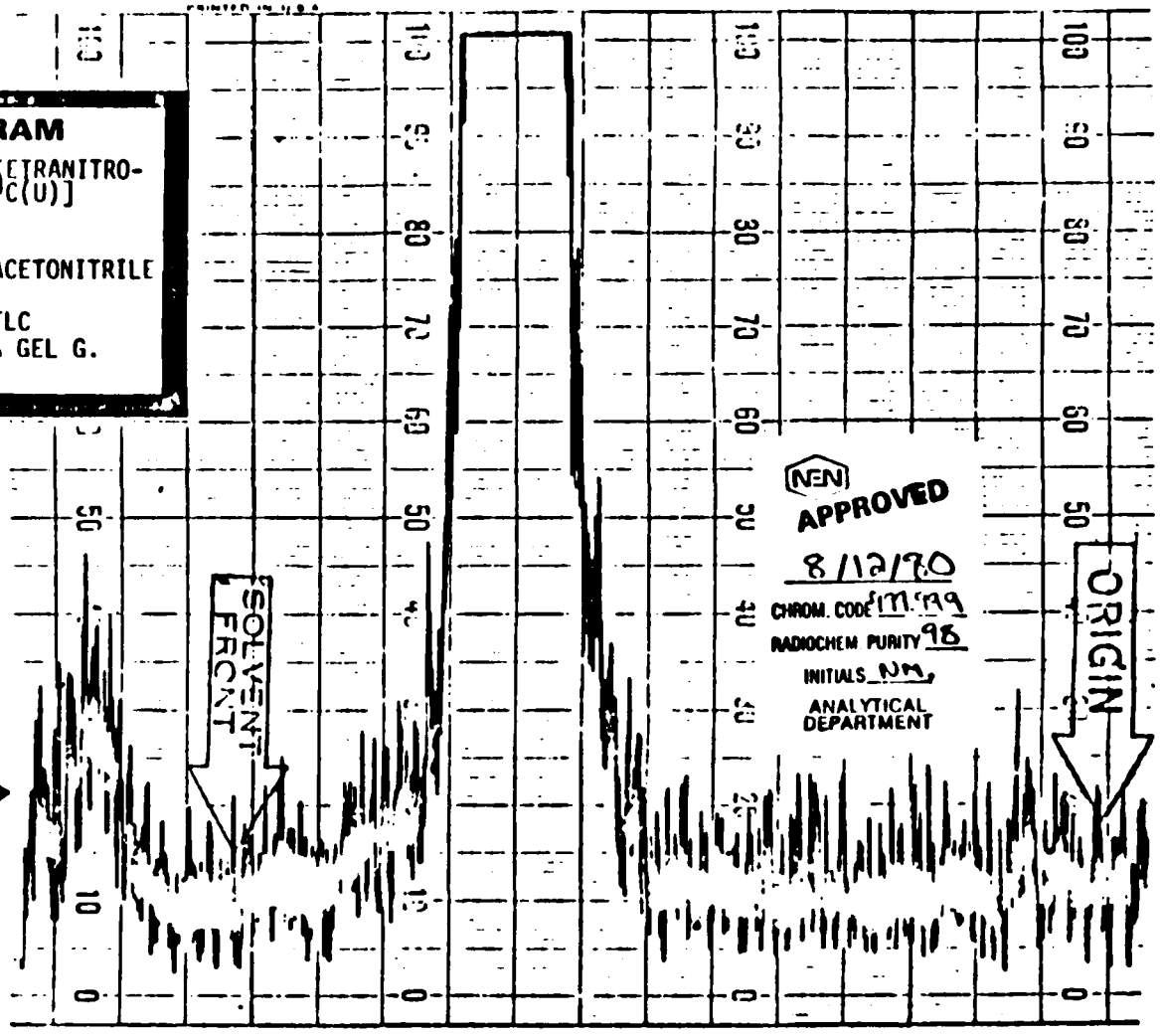
Compound: "HMX" OCTAHYDRO-1,3,5,7-TETRANITRO-1,3,5,7-TETRAZOCINE [^{14}C(U)]

Lot No: 1221-068

Solvent System: METHYLENE CHLORIDE:ACETONITRILE (16:6 V/V)

NEN New England Nuclear

TLC
SILICA GEL G.



RADIOCHEMICAL PURITY IS GREATER THAN 98%.

BASED ON COMPARATIVE PEAK AREA MEASUREMENT.

The sample was diluted 1 :100 and chromatographed with the undiluted material. The inserted trace represents an area equal to 1 % of the major peak. When compared to elevated areas of the baseline, it can be demonstrated that the total radiochemical impurities separated by this system are less than 2 %. The time constant, chart speed, slit width and full scale deflection is identical for both scans.

New England Nuclear

Radiochemical Specifications

PURITY VERIFICATION

Catalog Number: NEC-999H Lot Number: 1221-068

Compound: "HMX" OCTAHYDRO-1,3,5,7-TETRANITRO-1,3,5,7-TETRAZOCINE [^{14}C(U)]

The radiochemical purity of this compound was checked by thin layer chromatography using the solvent system listed below. The relative purity has been estimated after autoradiography by comparing the intensity of any impurities visible in the sample lane to either;

a. The intensity of 1% of the activity chromatographed in an adjacent lane, or
b. The intensity of 1% spotted on the plate above the solvent front.

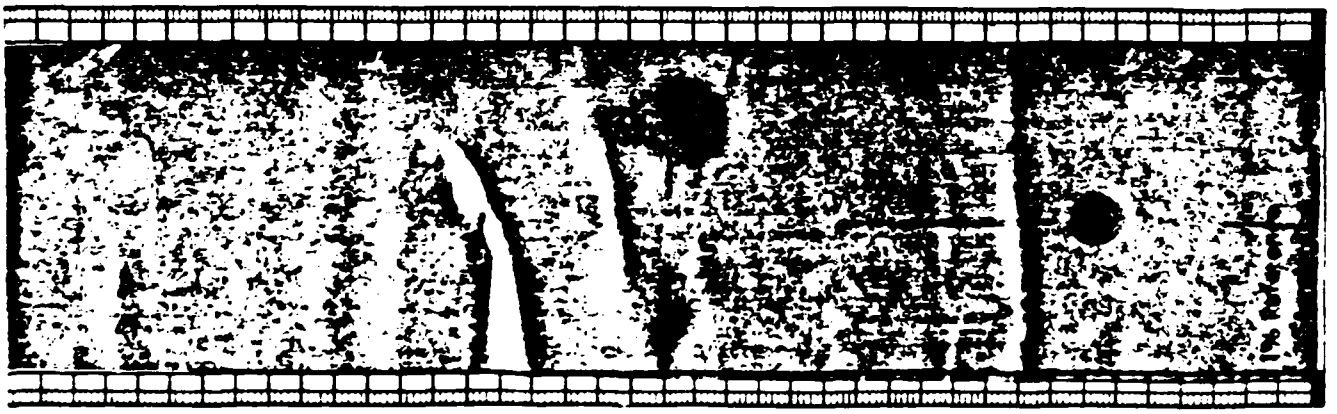
Media used: TLC SILICA GEL G.

Solvent system: METHYLENE CHLORIDE:ACETONITRILE (16:6 V/V)

Radiochemical purity is greater than 98 % as determined by the above method on 8/21/80.

SOLVENT FRONT

ORIGIN



1% REFERENCE STANDARD

1 division = 1 mm

APPENDIX 2

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Actual Oral Doses Administered to the Rat (Target Dose level was 500 mg.kg^{-1})

Animal No.	Body Weight (g)	Dose Received				Experimental Purpose
		dpm	µCi	mg	mg.kg^{-1}	
1♂	149	13949273	6.28	69.41	465.8	A
2♂	144				482.0	A
3♂	142				488.8	A
4♂	147				472.2	A
6♂	139				499.3	A
13♀	131				529.8	A
14♀	139				499.3	A
14♀	134				518.0	A
16♀	142				488.8	A
21♀	120				578.4	A
11♂	140	13949273	6.28	69.41	495.8	B
7♂	151				459.7	B
8♂	145				478.7	B
9♂	140				495.8	B
12A♂	129				538.0	B
24♀	122				568.9	B
19♀	135				514.1	B
20♀	134				518.0	B
23♀	137				506.6	B
22♀	139				499.3	B
25♂	154	15576187	7.02	77.50	503.3	C
26♂	153				506.6	C
27♂	133				582.7	C
28♂	151				513.3	C
29♂	164				472.6	C
30♂	165				469.7	C
31♀	140				553.6	C
32♀	132				587.1	C
33♀	135				574.1	C
34♀	142				545.8	C
35♀	136				569.9	C
36♀	144				538.2	C

A = Balance excretion study
B = Plasma levels of radioactivity
C = Terminal plasma levels of radioactivity

APPENDIX 3

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:

Actual Oral Doses Administered to the Mouse (Target Dose 500 $mg.kg^{-1}$)

Animal No.	Body Weight (g)	Dose Received dpm	µCi	mg	$mg.kg^{-1}$	Experimental Purpose	Animal No.	Body Weight (g)	Dose Received dpm	µCi	mg	$mg.kg^{-1}$	Experimental Purpose
1♂	21	2494986	1.12	12.41	591.2	A	25♂	22	2494986	1.12	12.41	496.6	B
2♂	23				539.8	A	*26♂	24				517.3	B
3♂	22				564.3	A	27♂	20				620.7	B
31♂	23				539.8	A	28♂	26				477.5	B
5♂	23				539.8	A	29♂	23				539.8	B
33♀	20	2095788	0.94	10.43	521.4	A	38♀	19	2095788	0.94	10.43	548.8	B
34♀	20				521.4	A	39♀	19				548.8	B
35♀	19				548.8	A	40♀	19				548.8	B
36♀	19				548.8	A	41♀	18				579.3	B
37♀	19				548.8	A	42♀	20				521.4	B
6♂	21	2494986	1.12	12.41	591.2	B	43♀	20				521.4	B
7♂	22				564.3	B	44♀	20				521.4	B
8♂	22				564.3	B	45♀	18				579.3	B
9♂	23				539.8	B	46♀	20				521.4	B
10♂	23				539.8	B	47♀	20				521.4	B
11♂	26				488/5	B	48♀	18				579.3	B
12♂	22				564.3	B	49♀	20				521.4	B
13♂	22				564.3	B	50♀	19				548.8	B
14♂	23				539.8	B	51♀	19				548.8	B
15♂	26				477.5	B	52♀	21				496.6	B
16♂	21				591.2	B	53♀	21				496.6	B
17♂	24				517.3	B	62♀	20				521.4	B
30♂	23				539.8	B	55♀	19				548.8	B
19♂	25				496.6	B	56♀	18				579.3	B
20♂	26				477.5	B	57♀	20				521.4	B
32♂	24				517.3	B	58♀	19				548.8	B
22♂	22				564.3	B	59♀	20				521.4	B
23♂	22				564.3	B	60♀	21				496.6	B
24♂	22				564.3	B	61♀	21				496.6	B

* = Animal No. 26♂ died at dosing A = Balance excretion study B = Terminal plasma level of Radioactivity

APPENDIX 4

The Pharmacokinetics of ^{14}C-HMX Following Oral Administration to the Rat and Mouse and Intravenous Administration to the Rat:
Actual Intravenous Doses Administered to the Rat (Target Dose 2 mg.kg^{-1})

Animal No.	Body Weight (g)	Dose Prepared dpm	Dose Residue dpm	Dose Received dpm	µCi	mg	mg.kg^{-1}	Experimental Purpose
46♂	140	10401794	226700	10175094	4.56	0.26	1.86	A
47♂	133		12090	10389704	4.68	0.271	1.99	A
50♂	154		90798	10310996	4.64	0.26	1.71	A,C
52♂	147		12712	10389082	4.68	0.27	1.80	A
54♂	141		11932	10389862	4.68	0.27	1.88	A,C
63♀	115		47900	10353894	4.66	0.26	2.30	A
64♀	120		12815	10388979	4.68	0.26	2.10	A
68♀	134		111335	10290459	4.64	0.26	1.96	A
69♀	137		15872	10385922	4.68	0.27	1.93	A,C
71♀	122		119445	10282349	4.63	0.26	2.13	A,C
43♂	150	11546500	54135	11492365	5.18	0.29	1.96	B
38♂	139		60753	11485747	5.17	0.29	2.11	B
39♂	144		45540	11500960	5.18	0.29	2.04	B
40♂	140		8155	11538345	5.20	0.29	2.10	B
41♂	131		22503	11523997	5.19	0.29	2.25	B
72♀	134	10401794	309205	10092589	4.55	0.26	1.92	B
62♀	142		39090	10362704	4.67	0.26	1.86	B
65♀	134		74995	10326799	4.65	0.26	1.97	B
59♀	139		83643	10318151	4.64	0.26	1.89	B
60♀	145		314103	10087691	4.54	0.26	1.78	B
78♂	143	11064315	*1372342	9691973	4.37	0.25	1.73	C
83♂	130		64760	10999555	4.96	0.28	2.16	C
84♂	140		1137373	9926942	4.47	0.25	1.81	C
94♂	149		2592200	8472115	3.82	0.22	1.45	C
86♀	139		266217	10798098	4.86	0.28	1.98	C
87♀	130		68933	10995382	4.95	0.28	2.16	C
89♀	130		*8848339	2215976	1.00	0.06	0.44	C
90♀	136		*1763749†	9300566	4.19	0.24	1.75	C

* = Includes contribution from tissue surrounding site of dosing

† = This animal received a dose of 37 µl via tail vein, all other animals received 30 µl via saphenous vein

A = Balance excretion study, B = Plasma levels of radioactivity, C = Terminal plasma levels of radioactivity

PERSONNEL INVOLVED IN PROJECT 415669PK AND AMENDMENTS 1, 2, 3, 4 AND 5

Principal Investigator:	A.B. Wilson, B.V.Sc., M.R.C.V.S., D.A.B.T.
Group Head, Laboratory Sciences:	G.H. Draffan, B.Sc., Ph.D.
Head, Metabolic Studies:	B.D. Cameron, B.Sc., M.I.Biol.
Scientists and Technical Assistance:	M.R. Currie, B.Sc., A.S. Givan J.C.T. Lang, B.Sc., Ph.D., M.I.Biol. J. McDougall, B.Sc., Ph.D. E.M. Sutherland, O.N.C. C.G. Young, B.Sc.
Quality Assurance:	A.W. Waddell, B.Sc., Ph.D. J.C. Brown, B.Sc., M.I.Biol. D. Watson, B.Sc. L. Nicol, B.Sc. C. Skakel, B.Sc.

DISTRIBUTION LIST

5 copies to:

Commander
U.S. Army Medical Research and Development Command
Attention: SGRD-RMS
Fort Detrick, Frederick, Maryland 21701-5012

6 copies to:

Inveresk Research International Limited
Musselburgh, EH21 7UB, Scotland